From Department of Clinical Science, Intervention and Technology
Division of Transplantation Surgery
Karolinska Institutet, Stockholm, Sweden

THE ROLE OF FIBROBLAST GROWTH FACTOR 19 IN HUMAN LIVER

Heléne Johansson

Stockholm 2020

Published by Karolinska Institutet.
Printed by E-print AB 2020

ISBN 978-91-7831-782-0

The role of Fibroblast Growth Factor 19 in human liver

THESIS FOR DOCTORAL DEGREE (Ph.D.)

By

Heléne Johansson

Principal Supervisor:
Assistant professor Ewa Ellis, PhD
Karolinska Institutet
Department of Clinical Investigation,
Intervention and Technology
Division of Transplantation Surgery

Co-supervisor(s):
Associate professor Greg Nowak, MD, PhD
Karolinska Institutet
Department of Clinical Investigation,
Intervention and Technology
Division of Transplantation Surgery

Associate professor Björn Fischler, MD, PhD
Karolinska Institutet
Department of Clinical Investigation,
Intervention and Technology
Division of Pediatrics

Professor Bo-Göran Ericzon, MD, PhD
Karolinska Institutet
Department of Clinical Investigation,
Intervention and Technology
Division of Transplantation Surgery

Opponent:
Assistant Professor Frank G Schaap, PhD
Maastricht University
Department of Surgery
The Netherlands

Examination Board:
Professor Ulf Diczfalusy, PhD
Karolinska Institutet
Department of Laboratory Medicine
Division of Clinical Chemistry

Professor Tomas Wester, MD, PhD
Karolinska Institutet
Department of Women's and Children's Health
Division of Pediatric Surgery

Associate professor Alireza Biglarnia, MD, PhD
Lund University
Department of Clinical Sciences
Division of Surgery

To my dad

ABSTRACT

Bile acids are synthesized in the liver and are essential for intestinal absorption of lipids. Bile acid homeostasis is crucial and if not maintained, bile acids can accumulate to toxic levels and cause severe damage to the liver. Bile acid homeostasis is therefore strictly regulated through feedback mechanisms where bile acids act as signaling molecules to control different aspects of their metabolism. The regulatory protein Fibroblast growth factor 19 (FGF19) has been of particular interest in recent years for its role in the negative feedback response of bile acid synthesis. FGF19 is induced in the terminal ileum in response to bile acids that are reabsorbed from the intestine. FGF19 is released to portal blood and will bind to its receptor in the liver to down regulate the rate-limiting enzyme in bile acid synthesis.

The studies in this thesis are focused on using human models to further understand the role of FGF19 in human bile acid metabolism in health and disease. We aimed to establish physiological levels of FGF19 and investigate how disease can change the dynamics of bile acids and FGF19. Furthermore, the response to FGF19 on a molecular level was studied in primary human hepatocytes.

In **Study I** we found that portal levels of FGF19 do no differ from levels in systemic blood under fasted conditions, which was in contrast to bile acids that had a higher concentration in portal blood compared to systemic blood due to an efficient uptake of the bile acids by the hepatocytes. Both bile acids and FGF19 increased postprandially, but no hepatic clearance of FGF19 was observed. A strong correlation between FGF19 and bile acids was observed, indicating that high levels of bile acids result in increased levels of FGF19.

In **Study II**, FGF19 and bile acids were investigated in cholestatic infants diagnosed with biliary atresia at time of Kasai portoenterostomy (KPE) surgery and at short-term follow-up. An adaptive response of the liver to increased hepatic bile acids and an interrupted enterohepatic circulation was observed at time of surgery, with reduced bile acid synthesis, increased levels of circulating bile acids and alterations in bile acid transporters. In addition, FGF19 levels were elevated and it was concluded that the surge in FGF19 originated from the liver in this condition. FGF19 levels had declined at follow-up and the decrease coincided with markers that indicate a restored bile flow, such as decreased levels of circulating bilirubin and conjugated chenodeoxycholic acid (CDCA). This might indicate that FGF19 could be a possible prognostic marker for outcome of KPE in biliary atresia.

In **Study III**, the response to the primary bile acid CDCA and FGF19 was investigated in primary human hepatocytes with special regard to bile acid synthesis. Physiological levels of CDCA as well as conditioned medium containing FGF19 produced by the hepatocytes, down regulated bile acid synthesis, while recombinant FGF19 did not. In addition, physiological levels of CDCA rapidly induced FGF19 gene expression in primary human hepatocytes, indicating a possible autocrine function for FGF19 in primary human hepatocytes. Bile acid synthesis was however efficiently down regulated by CDCA following siRNA knockdown of FGF19, suggesting that an autocrine pathway of FGF19 for down regulation of bile acid synthesis is unlikely.

In summary, no difference in concentration of FGF19 was observed between portal and systemic blood. Circulating FGF19 was elevated in cholestatic infants and originated from the liver. Physiological concentrations of CDCA induced FGF19 in primary human hepatocytes, however bile acid synthesis was efficiently down regulated independently of hepatic FGF19, presumably via the regulatory nuclear receptor small heterodimer partner (SHP). Contribution of hepatic FGF19 in regulation of bile acid synthesis may therefore be negligible under normal circumstances.

POPULÄRVETENSKAPLIG SAMMANFATTNING

Gallsyror tillverkas i levern och är livsnödvändiga för upptag av fettlösliga ämnen från tarmen, exempelvis fettlösliga vitaminer. Gallsyror fungerar som detergenter och bryter upp fettpartiklar till mindre enheter vilket är nödvändigt för att fett ska kunna passera tarmmembranet. De ingår i ett kretslopp som kallas för det enterohepatiska kretsloppet och cirkulerar från levern via gallblåsan till tarmen, där de återupptas och utsöndras till portablodet som för dem tillbaka till levern där de återigen tas upp och utsöndras till gallblåsan. Det enterohepatiska kretsloppet cirkulerar flera gånger per dag.

Produktion, utsöndring och upptag av gallsyror behöver vara i balans. Inte minst för att gallsyror i egenskap av detergenter i för hög dos kan vara toxiska för kroppens egna celler eftersom kroppens celler består av ett lipidmembran som kan luckras upp. Sjukdomar som orsakar rubbningar i gallsyracykeln orsakar även andra problem som exempelvis bristsjukdomar när vitaminer inte kan absorberas från tarmen. För att hålla balans i gallsyrasystemet fungerar gallsyror som signalsubstanser. Det betyder att gallsyrorna genom att aktivera olika reglerande faktorer kan stänga av sin egen nyproduktion samt reglera upptag och utsläpp från levern. Ett protein som anses ha en viktig roll i regleringen av nyproduktion av gallsyror är Fibroblast growth factor 19 (FGF19). FGF19 är ett protein med hormonliknande egenskaper, d.v.s. det kan utsöndras från en plats i kroppen och via blodcirkulationen ta sig till sitt målorgan där det utför sin uppgift. Forskning, som mestadels grundar sig på studier i möss, har visat att när gallsyrorna tas upp i tarmen signalerar de så att tarmcellerna börjar producera FGF19. FGF19 utsöndras sedan till portablodet och när det når levern signalerar det för att stoppa nyproduktion av gallsyror. Det här kallas för ett ”feedback-system” och är till för att hålla en konstant nivå av gallsyror.

Systemet för gallsyror skiljer sig mellan olika arter, vilket innebär att de slutsatser man drar i studier på t.ex. möss måste verifieras i människa. I det här arbetet har vi fokuserat på några av de frågorna som finns kring funktionen av FGF19 i regleringen av gallsyror hos människa.

I **Studie I** var vårt mål att bestämma de fysiologiska nivåerna av FGF19 i blod d.v.s. hur mycket FGF19 som faktiskt når levern när gallsyrorna signalerat för att det ska produceras. Den här informationen är viktig för vidare studier av proteinets påverkan på celler, men det är också viktigt för att identifiera vad som är normala nivåer och om/hur nivåerna skiljer sig vid sjukdom. Många ämnen som kommer från tarmen via portablodet till levern tas upp i levern och har därför en annan koncentration i portablodet jämfört med den systemiska cirkulationen. För att veta vilka nivåer av FGF19 som är fysiologiska mätte vi därför FGF19 i

portablod. Då portavenen ligger djupt inbäddad i magen kan detta bara göras under kirurgi. Vi samlade portablod från patienter som genomgick leverkirurgi och jämförde nivåerna av FGF19 i portablod med nivåerna i systemiskt blod taget vid samma tillfälle från ett blodkärl i armen eller på halsen. Slutsatsen var att nivåerna inte skiljde sig mellan portablod och systemiskt blod och FGF19 hos människa kan därmed mätas med ett vanligt blodprov.

I **Studie II** undersökte vi FGF19 hos små barn med sjukdomen gallvägsatresi, som innebär att galla inte kan transporteras från levern till tarmen. Orsaken är att gallgångarna som leder gallan från levern tillbakabildas efter födseln, men varför detta sker är oklart. Gallvägsatresi är en allvarlig sjukdom som innebär att levern utsätts för höga nivåer av gallsyror. Barn som lider av gallvägsatresi får svår klåda, blir gula och har svårt att ta upp näring från tarmen. De höga nivåerna av gallsyror skadar levern och för att överleva tvingas många genomgå levertransplantation vid tidig ålder. För att lindra symptomen och för att försöka undvika en transplantation utför man en så kallad Kasaioperation. En del av tarmen fästs då direkt till levern i ett försök att dränera galla till tarmen och på så sätt få igång ett enterohepatiskt kretslopp.

I den här studien undersökte vi hur nivåerna av FGF19 i blod skiljer sig hos barn med gallvägsatresi jämfört med friska barn samt om/hur FGF19 förändras efter Kasaioperation. Vi såg att FGF19 var kraftigt förhöjt hos barn med gallvägsatresi vid tidpunkten för Kasaioperation och till skillnad från friska individer utsöndrades FGF19 från levern. Detta är troligtvis orsakat av de onormalt höga nivåerna av gallsyror i levern som triggar samma system som de normalt sett gör i tarmen. Att FGF19 börjar produceras i levern förmodas vara en räddningsmekanism som syftar till att stänga ner all nyproduktion av gallsyror för att på så sätt hålla nere nivåerna. I enlighet med den hypotesen noterade vi att de markörer som finns för nyproduktion av gallsyror var lägre hos barn med gallvägsatresi än hos kontrollgruppen.

Mellan 4-6 månader efter operationen togs ett uppföljande blodprov från barnen med gallvägsatresi. Vi såg då att FGF19 sjunkit till nivåer som liknar dem hos friska barn. Vid en närmare undersökning såg vi att minskningen av FGF19 i blod var mer markant hos de barn som hade ett mer gynnsamt sjukdomsförlopp efter Kasaioperationen. Baserat på de här resultaten spekulerar vi att FGF19 skulle kunna användas som en markör för sjukdomsförloppet efter operation, men det kräver fler och större studier för att utreda om ett faktiskt samband finns.

I **Studie III** undersökte vi hur humana leverceller svarar på behandling av FGF19 på molekylär nivå i jämförelse med hur de svarar på gallsyror. Vi behandlade humana

leverceller med s.k. rekombinant FGF19 (framställt i bakterier) eller gallsyror i koncentrationer som kan räknas som fysiologiska enligt vår första studie. Försöken visade att rekombinant FGF19 hade en svag effekt på gallsyraproduktionen medan celler som behandlats med gallsyror hade en mycket lägre nyproduktion. Man vet sedan tidigare att det finns andra feedback-system i levern som triggas av gallsyror för att kontrollera nyproduktion. Detta visar att feedback-systemen i levern effektivt kan hålla en balans i tillverkningen av gallsyror även när FGF19 inte signalerar via tarmen. Anledningen till att rekombinant FGF19 inte har den förväntade effekten skulle kunna bero på framställningen av proteinet i bakterier som kan göra det mindre biologiskt aktivt, detta behöver dock studeras närmare. Det skulle även kunna bero på att FGF19 i själva verket har en annan primär funktion än reglering av gallsyraproduktionen. Vidare studier behövs därmed också för att kunna bedöma hur stor påverkan respektive system har på gallsyraproduktionen – feedback-systemet från tarmen och feedback-systemet i levern.

Något överraskande såg vi att FGF19 även producerades av levercellerna efter behandling med fysiologiska nivåer av gallsyror. Detta är något som man tidigare främst har sett i situationer med höga nivåer av gallsyror i levern, som beskrivet i studie II. Vi testade därför om de nivåer av FGF19 som bildades av levercellerna hade effekt på gallsyraproduktionen och därmed kan vara en bidragande anledning till varför nyproduktionen var lägre efter behandling med gallsyror i vårt första försök. Vidare försök visade dock att nivåerna av FGF19 som bildades inte var tillräckliga för att ha någon effekt på nyproduktion av gallsyror. Från resultaten i den här studien drar vi slutsatsen att gallsyror, oberoende av FGF19, effektivt kan reglera sin egen produktion via andra regulatoriska faktorer i levern. De höga nivåerna av FGF19 som bildas i levern vid sjukdom kan dock vara av klinisk betydelse för reglering av nyproduktion under extrema förhållanden.

Sammanfattningsvis har vi i det här arbetet visat vad de fysiologiska nivåerna av FGF19 är och hur nivåerna förändras vid sjukdomar som påverkar systemet för gallsyror. Vidare har vi visat indikationer på att FGF19 följer sjukdomsförloppet, vilket skulle kunna göra FGF19 till en markör för förloppet hos den här typen av sjukdomar. Detta kräver dock fler studier. Rekombinant FGF19 har svag effekt på humana levercellers produktion av gallsyror. Gallsyror kan inducera FGF19 i leverceller men under normala förhållanden är nivåerna troligtvis för låga för att ha en effekt på nyproduktion av gallsyror och gallsyrorna kan själva nedreglera sin nyproduktion i levern oberoende av FGF19. Vidare studier av FGF19 i system som bygger på humana modeller krävs för att fullständigt förstå vilken roll FGF19 har i regleringen av gallsyror både under normala förhållanden och vid sjukdom.

LIST OF SCIENTIFIC PAPERS

I. **Circulating Fibroblast growth factor 19 in portal and systemic blood.**
Johansson H, Mörk L-M, Li M, Sandblom AL, Björkhem I, Höijer J, Ericzon B-G, Jorns C, Gilg S, Sparrelid E, Isaksson B, Nowak G, Ellis E.
J Clin Exp Hepatol. 2018;8:162-68.

II. **Regulation of bile acid metabolism in biliary atresia: Reduction of FGF19 by Kasai portoenterostomy and possible relation to early outcome.**
Johansson H, Svensson JF, Almström M, Van Hul N, Rudling M, Angelin B, Nowak G, Fischler B and Ellis E.
J Intern Med. 2020 doi: 10.1111/jpim.13028.

III. **Chenodeoxycholic acid modulates bile acid synthesis independent of Fibroblast growth factor 19 in primary human hepatocytes.**
Johansson H, Nørskov Søndergaard J, Jorns C, Kutter C, Ellis E.
Manuscript 2020.

CONTENTS

LIST OF ABBREVIATIONS

ASBT	Apical sodium-dependent bile salt transporter
BAAT	Bile acid-CoA:amino acid N-acyltransferase
BDL	Bile duct ligation
BSEP	Bile salt export pump
C4	7α-4-cholesten-3-1
CA	Cholic acid
CDCA	Chenodeoxycholic acid
CK7	Cytokeratin 7
CYP7A1	Cholesterol 7α-hydroxylase
CYP27A1	Sterol-27 hydroxylase
CYP7B1	Oxysterol 7α-hydroxylase
CYP8B1	12α-hydroxylase
DCA	Deoxycholic acid
FGF15/19/21/23	Fibroblast growth factor 15/19/21/23
FGFR	Fibroblast growth factor receptor
FXR	Farnesoid X receptor
GC-MS	Gas chromatography-mass spectrometry
HCC	Hepatocellular carcinoma
HNF4α	Hepatocytes nuclear factor 4α
IBABP	Ileal bile acid binding protein
KPE	Kasai portoenterostomy
LC-MS/MS	Liquid chromatography-tandem mass spectrometry
LCA	Lithocholic acid
LXRα	Liver X receptor
MDR3	Multidrug resistance protein 3
MRP2	Multidrug resistance-associated protein 2
NTCP	Sodium-dependent taurocholate co-transporting polypeptide
OATP	Organic anion-transporting polypetides
OSTα/β	Organic solute transporter
OCA	Obeticholic acid
PBC	Primary biliary cholangitis
PFIC2	Progressive familial intrahepatic cholestasis type 2
RXR	Retinoid X receptor
TGR5	G protein-coupled bile acid receptor
UDCA	Ursodeoxycholic acid

1 INTRODUCTION

1.1 LIVER ANATOMY AND PHYSIOLOGY

The liver is a central organ for maintaining glucose and lipid homeostasis, for bile acid metabolism and for detoxification of endogenous and exogenous substances. Low-oxygenated portal venous blood account for 80 percent of the blood supply to the liver while the remaining 20 percent is oxygenated blood provided by the hepatic artery. Blood flows through the sinusoids of the liver and eventually drains into the central vein. Portal blood brings nutrients and other substances from the intestine to be metabolized and distributed to other tissues and organs or eliminated from the body [1,2]. Hepatocytes, the parenchymal cells of the liver, are polarized epithelial cells with the basal side facing the endothelium of the sinusoids. The apical surface forms bile canaliculi where bile components are secreted into a network of channels that carry bile to the bile ducts. On the lateral side, between adjacent hepatocytes, are gap junctions for cell-cell interactions and tight junctions to seal off the bile canalicular lumen. This gives the hepatocytes a structural pattern of a hexagonal unit called a hepatic lobuli with rows of 15-25 hepatocytes residing adjacent from the portal triad, made up by a portal vein, hepatic artery and bile duct, to the central vein (Figure 1) [1–3]. Non-parenchymal cells in the liver include cholangiocytes, endothelial cells, Kupffer cells, hepatic stellate cells and NK-cells [1,2,4].

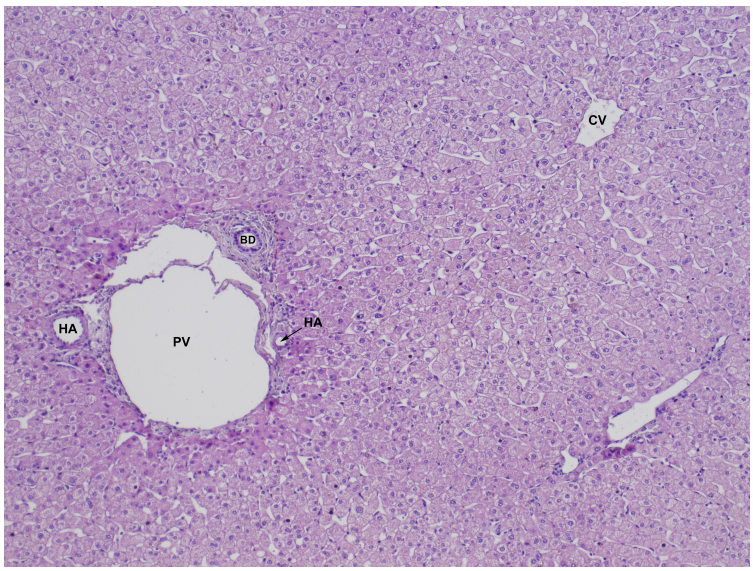

Figure 1. Haemotoxylin and eosin staining of liver section at 20x magnification showing a portal triad (PV=portal vein, HA=hepatic artery, BD=bile duct) and central vein (CV). Blood flows from the portal vein and hepatic arteries through the sinusoids between rows of hepatocytes towards the central vein. Bile flows in the opposite direction through bile canaliculi and drains into the bile duct.

1.2 BILE ACIDS

The transformation of cholesterol into bile acids by hepatocytes is one of the major routes for eliminating cholesterol from the body. Bile acids are essential for absorption of lipids in the intestine but have also proved to be important signaling molecules by activation of the nuclear receptor Farnesoid X receptor (FXR) and the G protein-coupled bile acid receptor (TGR5) [5–7]. Via these receptors, bile acids regulate their own metabolism [8–11] as well as liver regeneration [12] and glucose and lipid metabolism [13].

1.2.1 Bile acid synthesis

Approximately 500 mg cholesterol per day is converted into primary bile acids in adult humans. There are two pathways for *de novo* synthesis of bile acids: the classic pathway, accounting for about 90 % of the production, and the alternative pathway. Biosynthesis of bile acids involves 17 different enzymes and takes place in different compartments of the cell: the cytosol, endoplasmic reticulum, mitochondria and peroxisomes [13–18].

The first step of the classic pathway is 7α-hydroxylation of cholesterol into 7α-hydroxycholesterol, catalyzed by the rate-limiting enzyme in bile acid synthesis, cholesterol 7α-hydroxylase (CYP7A1). Transcriptional inhibition of this enzyme is fundamental for regulation of bile acid synthesis in humans. 7α-hydroxycholesterol is then transformed into 7α-4-cholesten-3-1 (C4) by the isomerase/oxidoreductase HSD3B7. In the subsequent step of bile acid synthesis, the C4 intermediate can either be further hydroxylated by the introduction of a 12α-hydroxyl group catalyzed by 12α-hydroxylase (CYP8B1) which will then generate the final product of trihydroxylated cholic acid (CA). In the absence of 12α-hydroxylation, dihydroxylated chenodeoxycholic acid (CDCA) will instead be formed [14–16,19].

In the alternative pathway, the cholesterol side chain is hydroxylated into 27α-hydroxycholesterol catalyzed by mitochondrial sterol-27 hydroxylase (CYP27A1) and further hydroxylated by oxysterol 7α-hydroxylase (CYP7B1) [13,14]. While the classic pathway produces approximately twice as much CA as CDCA, the alternative pathway almost exclusively produces CDCA [14,16,20]. Before bile acids are released from the hepatocytes, they are conjugated with glycine or taurine catalyzed by bile acid coenzyme bile acid-CoA:amino acid N-acyltransferase (BAAT) to increase solubility and decrease toxicity [15,21]. While glycine conjugates dominates in adults, the majority of the bile acids in infants are conjugated to taurine [22,23]. In the intestine, CA and CDCA can be deconjugated and bacterial 7α-dehydroxylase then removes the 7α-hydroxyl group to form deoxycholic acid

(DCA) and lithocholic acid (LCA) respectively [14,16]. CDCA may also form an intermediate that is further modified into ursodeoxycholic acid (UDCA) [20]. Bile acid composition and levels differ in infants compared to adults. In addition to a different conjugation pattern, levels are elevated during the first months of life compared to adults and the composition is mostly dominated by primary bile acids due to an immature intestinal micro flora [24,25]. The key steps of bile acid synthesis are illustrated in Figure 2.

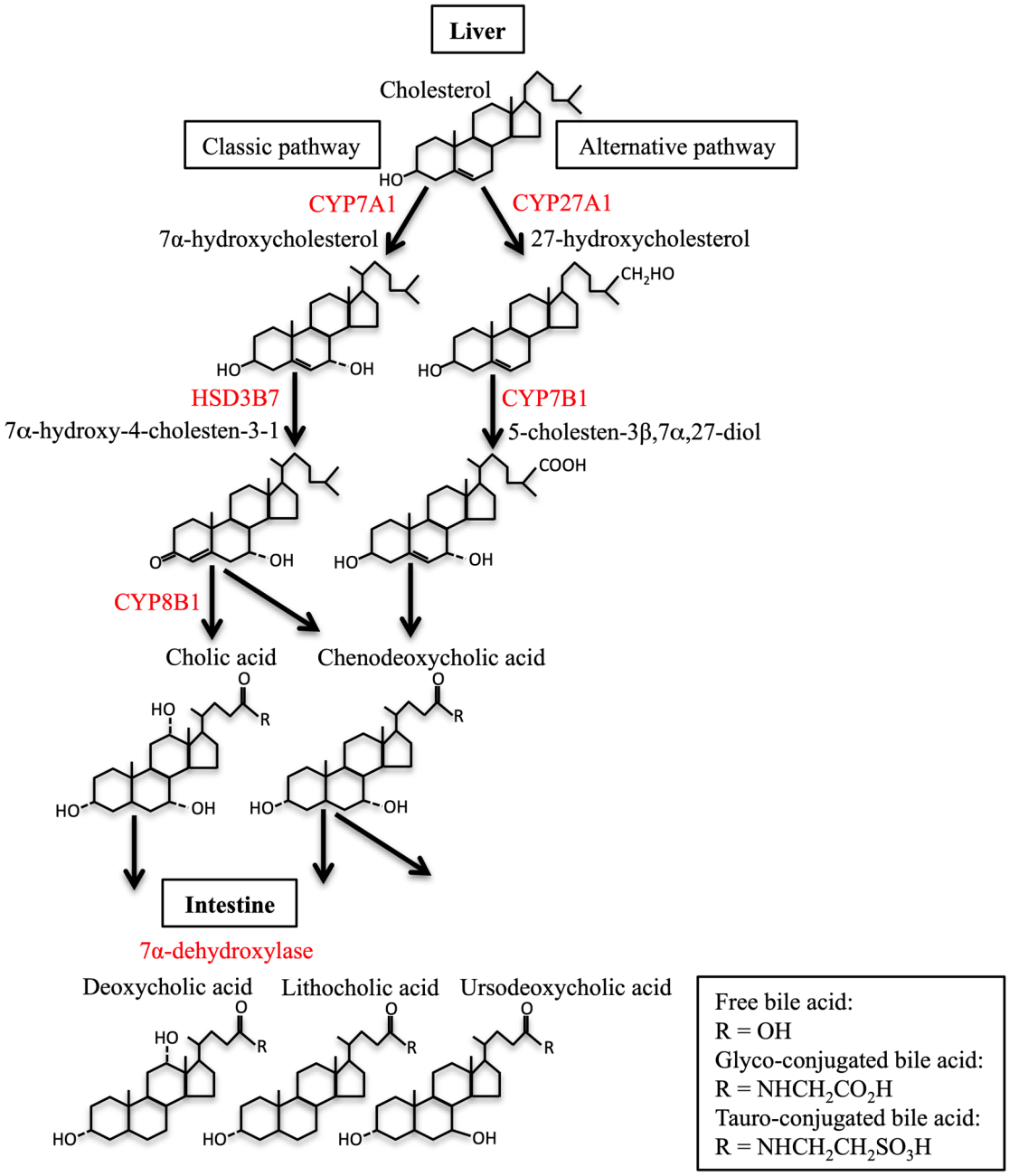

Figure 2. The key steps of bile acid synthesis: the classic pathway, the alternative pathway and conversion of primary bile acids to secondary bile acids in the intestine.

1.2.2 Enterohepatic circulation

The enterohepatic circulation is a highly efficient system that relies on active transport and passive diffusion. Bile acids are secreted from the liver to bile, reabsorbed in the intestine and transported back to the liver with a loss of approximately 5 percent. Conjugated bile acids are released from the hepatocytes into bile canaliculi through the bile salt export pump (BSEP) [26,27]. The importance of BSEP is demonstrated in patients with mutations in ABCB11, the gene encoding BSEP, which causes the severe cholestatic liver disease progressive familial intrahepatic cholestasis type 2 (PFIC2) [28,29]. In addition, the canalicular surface of hepatocytes includes transport systems for *e.g.* secretion of phospholipids (multidrug resistance protein 3, MDR3), bilirubin (multidrug resistance-associated protein 2, MRP2) and cholesterol (ATP-binding cassette sub-family G member 5 and 8, ABCG5/8) [26]. Bile acids and other components of bile are transported to the gallbladder where bile is stored and concentrated prior to being released into the intestine [30]. The majority of the bile acids are reclaimed at the terminal ileum via the apical sodium-dependent bile salt transporter (ASBT). In the enterocytes, bile acids bind to the cytosolic ileal bile acid binding protein (IBABP) and are excreted into portal blood via the sodium-independent transporters, organic solute transporter α and β heterodimer (OSTα/OSTβ) [26,27]. Bile acids that escape this route will continue to the colon where secondary bile acids are formed through the action of intestinal bacteria. Bile acids can be reabsorbed from the colon through passive diffusion. Bile acids return to the liver via the portal vein and are again efficiently reabsorbed with a small portion spilling over to the systemic circulation. Conjugated bile acids are reabsorbed through the sodium-dependent taurocholate co-transporting polypeptide (NTCP) while unconjugated bile acids are taken up by organic anion-transporting polypetides (OATPs) or diffusion, thus completing the enterohepatic circulation [27]. CA appears to be more efficiently cleared than CDCA and DCA resulting in different patterns of bile acid composition between portal and systemic blood [31,32]. Deconjugated bile acids are re-conjugated and again released to bile together with newly synthesized bile acids. Bile acids complete several circulations between the liver and intestine per day. Uptake, conjugation and secretion of bile acids by the hepatocytes is an efficient system that ensures maintenance of intracellular levels of bile acids to a level that does not become toxic for the cell. Hepatocytes can also secrete bile acids back to the sinusoids, this process becomes of importance during diseases that causes high intrahepatic levels of bile acids, *i.e.* cholestatic liver diseases. Important transporters on the sinusoidal surface for secretion of bile acids are OSTα/OSTβ, MRP3 and MRP4 [26,27]. The enterohepatic circulation is summarized in Figure 3.

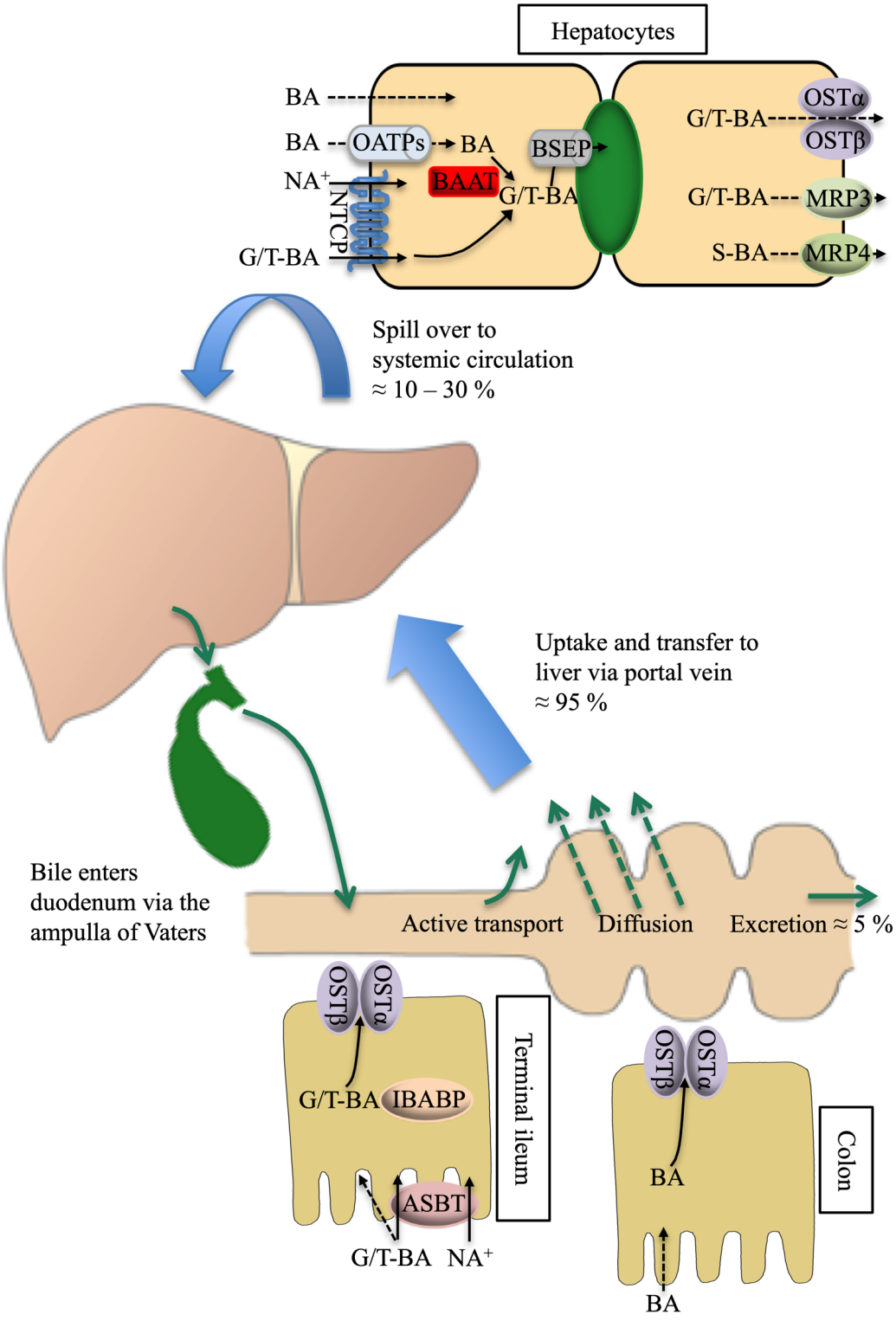

Figure 3. The enterohepatic circulation rotates between liver and intestine several times per day. Active transport and passive diffusion of bile acids in both liver and intestine ensures that only a small fraction needs to be replaced by *de novo* synthesis.

1.2.3 Regulation of bile acid metabolism

As powerful detergents, bile acids can be toxic and their metabolism needs to be strictly regulated to prevent cell damage. Most aspects of bile acid metabolism, synthesis, hepatic secretion, ileal transit as well as hepatic uptake, are therefore under rigorous control through feedback systems where FXR is a master regulator at transcriptional level [11,33–36]. The importance of FXR in regulation of bile acid synthesis has been demonstrated in mice where Fxr-null mice exhibit increased levels of bile acids, cholesterol, triglycerides and phospholipids in serum and loss of feedback regulation of Cyp7a1 [8]. FXR also controls the enterohepatic circulation and intracellular levels of bile acids by inducing transcription of BSEP in hepatocytes as well as ileal IBABP and OSTα/OSTβ, while repressing NTCP gene expression [34–36].

FXR forms heterodimers with the retinoid X receptor (RXR) and is in its inactivated form bound to the hormone response elements in the promoter region on target genes together with co-repressor proteins [37–39]. Bile acids are ligands for FXR and upon binding they cause a conformational change that results in the release of co-repressors and recruitment of co-activator proteins and thus induces transcription [6,7,38–40]. The strongest activator of FXR among the endogenous bile acids is CDCA, followed by DCA and CA [10,40], while the semi-synthetic analogue of CDCA, obeticholic acid (OCA), exhibits a nearly 100-fold greater capacity in activating FXR [41].

A target gene for FXR with a prominent role in bile acid metabolism is NR0B2, encoding the nuclear receptor small heterodimer partner (SHP). SHP is a non-typical nuclear receptor that does not bind directly to DNA due to its lack of a DNA-binding domain. Instead it interacts with other nuclear receptors and inhibits their action [11,42]. SHP is involved in transcriptional inhibition of NTCP in hepatocytes and ASBT in enterocytes and in suppression of bile acid synthesis by inhibition of CYP7A1 gene expression [11,36,43]. Its role in suppressing bile acid synthesis was discovered when primary human hepatocytes treated with an FXR-ligand displayed an increased expression of SHP, while the expression of CYP7A1 decreased in a dose-dependent manner. It was found that SHP interacts with and inhibits the actions of the nuclear receptors liver receptor homolog 1 (LRH-1) and hepatocyte nuclear factor 4α (HNF4α), two transcription factors that induce CYP7A1 (Figure 4) [11,44,45]. The importance of SHP has been demonstrated in mice with a disrupted Shp-gene; Shp-null mice have up to 50 percent increased expression of Cyp7a1 mRNA and protein, and an increased bile acid pool correlating to the increase of enzyme expression [46]. However, these studies also indicate that the FXR-SHP-LRH1/HNF4α cascade may not be

the only regulatory system for bile acid synthesis. Mice that lack Fxr have a higher gene expression of Cyp7a1 compared to Shp-null mice, indicating that an Fxr-dependent pathway independent of Shp exists for down regulation of Cyp7a1. In addition, CA still represses Cyp7a1 in Shp-null mice [46,47], altogether supporting the hypothesis that other mechanisms for regulating bile acid synthesis in mice exists.

1.3 FIBROBLAST GROWTH FACTOR 19

Fibroblast growth factor 19 (FGF19) is a member of the FGF family that includes 22 proteins in man [48,49]. FGFs, with the exception of FGF11-14, bind to plasma tyrosine receptor kinases called fibroblast growth factor receptors (FGFR) and typically act in an autocrine/paracrine manner. Most FGFs bind heparin or heparin sulfate proteoglycans, which stabilizes the interaction with the receptor and prevent them from diffusing from their target [49,50]. The subfamily of FGF19 (including FGF21, FGF23 and the FGF19 mouse ortholog Fgf15) has low affinity for heparin, which allows them to diffuse from their expression site and act as endocrine molecules by travelling through the blood circulation. At their target site, this subfamily of FGFs bind to their respective receptor only in the presence of tissue-specific single pass transmembrane proteins called αKlotho and βKlotho that will stabilize the interaction between the protein and the receptor [50–53]. FGF19 binds to the ubiquitously expressed receptor FGFR4 and requires βKlotho to stabilize the interaction [52–54]. βKlotho is found in adipose tissue, pancreas, intestine, gallbladder and liver [52,55].

FGFs are generally known for playing a role in embryogenesis [50]. However, the subfamily of FGF19/21/23 differs also in this aspect, as they are involved in different metabolic pathways. FGF21 is involved in fatty acid and glucose metabolism and FGF23 regulates phosphatase and Vitamin D metabolism in the kidney [50,56]. FGF19 has also been proposed to have a role in glucose and lipid metabolism as it was found that transgenic mice expressing human FGF19 had an increased metabolic rate and did not become obese or diabetic on a high fat diet. Moreover, administration of FGF19 normalized glucose levels and reduced gain weight in a model of obese, diabetic mice [57,58]. Furthermore, administration of FGF19 to mice stimulates hepatic protein and glycogen synthesis and inhibits hepatic gluconeogenesis [59,60]. However, the most prominent role of FGF19 appears to be in bile acid metabolism.

1.3.1 FGF19 in the regulation of bile acid synthesis

The human FGF19 and mouse Fgf15 gene are considered to be orthologs. They share approximately 50 percent amino acid sequence and have similar tissue expression pattern [61–63]. FGF19 and Fgf15 have been proposed to be key regulators of bile acid synthesis, acting as endocrine molecules originating from the intestine and acting independently of the hepatic FXR-SHP- LRH1/HNF4α pathway. Bile acids reabsorbed at the terminal ileum activate intestinal FXR that in turn induces gene expression of FGF19/15. The proteins are released from the enterocytes to the portal vein and inhibit CYP7A1 transcription by binding to FGFR4 and signaling through a MAPK/ERK1/2 pathway (Figure 4) [64–66].

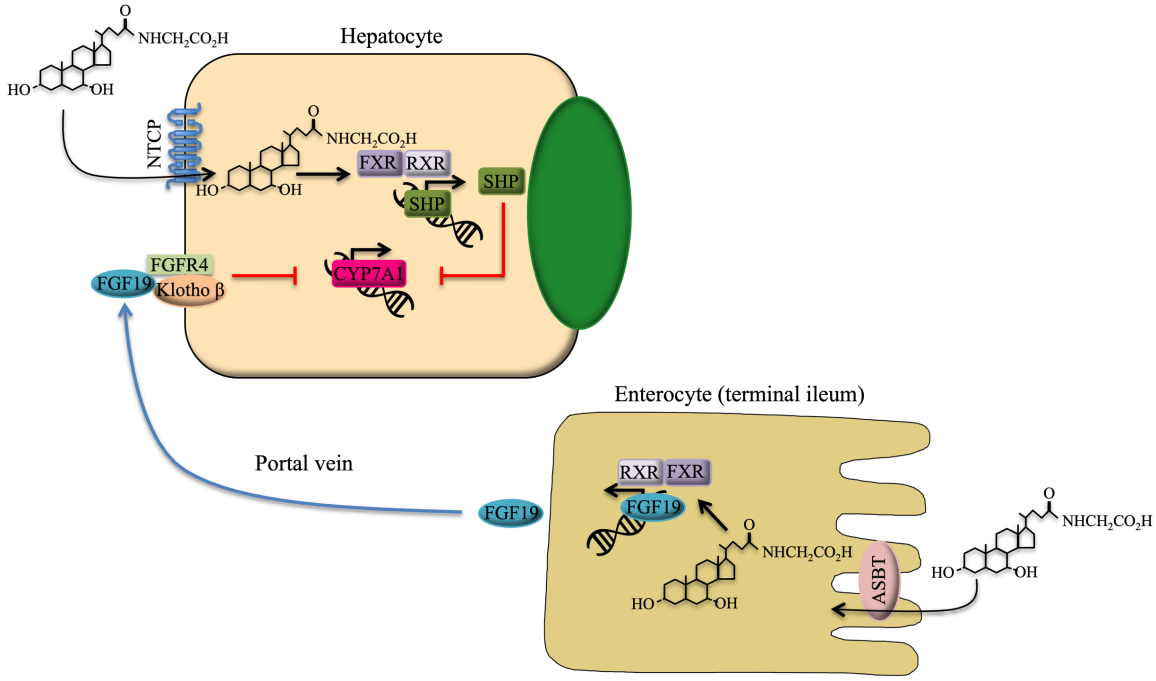

Figure 4. Schematic overview of the hepatic and intestinal pathway for regulation of bile acid synthesis. Bile acids returning to the liver activate hepatic FXR, which in turn induce expression of transcription factor SHP. SHP in turn down regulates CYP7A1. Bile acids reabsorbed at terminal ileum activate intestinal FXR which induce expression of FGF19. FGF19 is released to the portal blood stream and down regulates CYP7A1 by binding and signaling through FGFR4 and βKlotho.

An intestinal factor for mediating regulation of bile acid synthesis was first suggested when it was found that transcriptional inhibition of Cyp7a1 failed in rats infused intravenously with tauro-conjugated CA, while infusion intraduodenally efficiently reduced the gene expression [67]. It was then shown that mice with a knockout of the Fgfr4 gene had an elevated expression of Cyp7a1 and an increased bile acid pool, indicating a role for the receptor and its ligand in bile acid synthesis [68]. Holt *et al.* 2003 [65] reported that primary human hepatocytes treated with an FXR agonist expressed FGF19 and that high doses of recombinant FGF19 down regulated CYP7A1 in the cells. In addition, a response element for FXR was found in the FGF19 gene [65]. However, gene expression of FGF19 in healthy human liver turned out to be low or undetectable [61,69]. Likewise, Fgf15 gene expression could not be detected in mouse liver even after treatment with an FXR agonist, but was instead found in the small intestine with a particularly strong expression at terminal ileum. Furthermore, bile duct ligation (BDL) in mice, preventing bile to circulate from the liver to the intestine, resulted in a 3-fold increase in Cyp7a1 expression. Administration of FGF19 to the BDL mice on the other hand, suppressed Cyp7a1 gene expression [66], altogether supporting the notion of FGF19/15 being a regulator of bile acid synthesis acting through a bile acid induced feedback mechanism from the intestine.

Much of the current understanding of FGF19 in regulation of human bile acid synthesis has been deduced from studies in mice. A relationship between FGF19 and bile acids has also been indicated in humans. Systemic blood levels of FGF19 follows the diurnal rhythm of bile acids and peak approximately two hours after the postprandial peak of bile acids, in coherence with the concept that FGF19 is released from the intestine in response to a postprandial increase in the transintestinal bile acid flux [70–72]. There is also an inverse correlation between FGF19 and C4, an intermediate in bile acid synthesis that is found in the blood circulation and can be used as a measurement of bile acid synthesis, in agreement with a possible down regulation of bile acid synthesis by FGF19 [73]. However, the transintestinal flux of bile acids, inducing the intestinal pathway (FXR-FGF19-FGFR4/βKlotho) and then the hepatic pathway (FXR-SHP- LRH1/HNF4α), makes it difficult to assess the impact of each pathway on bile acid synthesis because of the time overlap. Moreover, profound species differences in bile acid metabolism between humans and rodents with substantial differences in bile acid composition and regulatory systems warrant extra caution when extrapolating animal data to human physiology. Rats for example, exhibit a feed forward mechanism of bile acid synthesis by the nuclear receptor Liver X receptor α (LXRα), while stimulation of

LXRα in primary human hepatocytes has the opposite effect with repression of CYP7A1 [74–76].

The understanding of FGF19's role in bile acid synthesis is further complicated by studies with sometimes-conflicting data. In studies on primary human hepatocytes, supra-physiological doses of FGF19 are required to suppress CYP7A1. Blood concentrations of FGF19 in healthy subjects are in the range of 50-150 pg/mL during fasting and increases up to four times following a meal [70–72]. An efficient suppression of CYP7A1 by recombinant FGF19 requires concentrations greater than 5000 pg/mL in primary human hepatocytes [64,65]. In contrast, physiological concentrations of bile acids efficiently suppress bile acid synthesis in cultures of primary human hepatocytes [31,65,77]. High doses of FGF19/15 have also been used in studies with mouse models, doses that would correspond to levels more than 60 times higher than what is found in the human circulation. That makes it difficult to distinguish a physiological response from effects observed due to supra-physiological conditions [56]. These are discrepancies that need to be addressed in order to fully understand the role of FGF19 as a regulator of human bile acid synthesis and necessitate studies in human models.

1.3.2 FGF19 in cholestatic liver diseases

Cholestasis is defined by a blockage of bile flow from the liver to the intestine. The consequence is a disrupted enterohepatic circulation resulting in a reduced or attenuated bile flow to the intestine, which consequently leads to malabsorption of essential nutrients. The restricted transport of bile acids from the liver leads to accumulation of bile acids to toxic levels in the liver and systemic circulation causing hepatic inflammation and cell damage. Chronic cholestasis increases the risk of hepatocellular and cholangiocellular carcinomas and eventually leads to liver fibrosis, cirrhosis and liver failure [14,78]. Cholestasis leads to adaptive changes in the liver that aim to reduce liver injury by the accumulated bile acids. FXR has a central role in these events by inducing efflux transporters to the systemic circulation (OSTα/OSTβ) while simultaneously blocking transcription of uptake transporters NTCP and OATPs, to reduce intracellular bile acid levels. Human cholestasis is further characterized by reduced bile acid synthesis demonstrated by low expression of CYP7A1 and the circulating bile acid intermediate C4. [14,69,78,79].

In cholestatic liver diseases, FGF19 is expressed also in the liver. In addition, circulating levels of FGF19 become elevated in direct correlation to the hepatic expression of FGF19. These events have been suggested to be part of the adaptive response to reduce toxic levels of

bile acids by reducing *de novo* synthesis [69,80,81]. It is generally assumed that elevated levels of intrahepatic bile acids, and subsequent activation of FXR, is responsible for the increase in FGF19. This is supported by reports on primary human hepatocytes that respond to bile acids by expressing FGF19 [64,65,69,81].

1.3.3 FGF19 expression and possible role in the gallbladder

FGF19 is highly expressed in the human gallbladder epithelial cells and is also, though to a lesser extent than in the gallbladder, expressed in the common bile duct. Furthermore, the concentration of FGF19 in bile can be up to a 100 times higher than in peripheral blood [55]. A role for FGF19 in regulation of gallbladder filling and relaxation has been proposed based on a mouse model lacking Fgf15 that had a virtually empty gallbladder that was rapidly filled with bile following injection of FGF19 [82]. If FGF19 from the gallbladder contributes to circulating FGF19 levels is currently not known. No long-term effect on blood levels of FGF19 following removal of the gallbladder has been observed. There is however an initial decrease of circulating FGF19 up to three months after cholecystectomy, which could indicate that circulating FGF19 is partly a spillover from synthesis in the gallbladder [73,83]. The normalization of FGF19 serum levels later on could reflect adaptive responses. The common bile duct has been known to dilate after the gallbladder is removed and it has been speculated that it becomes a reservoir for bile [84]. The readjustment by the bile duct into a gallbladder-like reservoir could possible lead to other gallbladder features such as increased FGF19 expression [55]. Its function in the human gallbladder, however, remains unknown at the moment and requires further studies.

1.4 BILIARY ATRESIA AND KASAI PORTOENTEROSTOMY

In Study II, we studied children with the cholestatic liver disease biliary atresia. Biliary atresia is a severe disease of unknown etiology characterized by obliteration of intrahepatic and/or extrahepatic bile ducts. It affects 1:10000 to 1:15000 newborns every year and typically presents at or shortly after birth. Left untreated the disease eventually progresses to liver failure and death before two years of age [85–89]. First-line treatment is the surgical procedure called Kasai portoenterostomy (KPE) that aims to restore bile flow to the intestine. In this procedure a Roux-en-y loop of the intestine is anastomosed to the liver [90]. KPE resolves cholestasis in about half of the cases, but the majority of the children require liver transplantation before adulthood [85,86,89,91].

2 AIMS

The general aim of this thesis was to investigate the role of FGF19 in human liver and specifically its role in bile acid homeostasis. To gain further understanding of FGF19 in health and in cholestatic liver disease we investigated:

Study I: If the concentration of FGF19, similar to bile acids and insulin, is higher in portal blood than the concentration of FGF19 in systemic blood under fasted and postprandial conditions.

Study II: If circulating levels of FGF19 changes between the time of KPE surgery in patients with biliary atresia and the time of follow-up 4-6 months post-surgery and how the levels at the respective time point compares to circulating levels of FGF19 in non-cholestatic infants.

Study III: How primary human hepatocytes respond to physiological concentrations of CDCA and FGF19 respectively and how they each impact bile acid synthesis.

3 MATERIAL AND METHODS

3.1 ETHICAL CONSIDERATIONS

All studies were approved by the Swedish ethics review authority in Stockholm and were in accordance with guidelines of the Helsinki Declaration. Written consent was obtained from the subjects included in the studies (Study I and III) or from their legal guardians (Study II).

3.2 SUBJECTS, SAMPLING AND STUDY DESIGN

Study I: The study was divided into two parts. For the first part of the study, blood samples (portal, peripheral arterial and central venous blood) were collected from 75 patients undergoing liver surgery. The patients were fasted for at least 6 hours prior to surgery. For the second part of the study, three brain-dead organ donors were included. Following collection of peripheral arterial and central venous blood samples (baseline) a 200 mL nutrient drink á 400 kcal (Resource 2.0, Nestlé) was administrated through a ventricle tube. Two hours after ingestion, during organ harvesting, blood samples were collected (portal, peripheral arterial and central venous blood). In addition, ten healthy volunteers were enrolled to control for a postprandial response post ingestion of the nutrient drink. Peripheral vein blood was drawn after an overnight fast (baseline) before intake of the nutrient drink and blood samples were collected every hour for the following four hours. Blood samples were centrifuged to retrieve the serum fraction and the samples were stored at -80°C.

Study II: Blood samples and liver and gallbladder biopsies were collected from 15 patients diagnosed with biliary atresia at time of surgery (Kasai portoenterostomy – KPE). Portal blood was retrieved from 12 of the patients by inserting a catheter through the umbilical vein during surgery. A follow-up blood sample was obtained during regular clinical check-ups 4-6 months following surgery. Two control groups were enrolled in the study. To control for changes induced by the surgical bowel reconstruction, blood samples were collected from five children undergoing KPE due to choledochal malformations and a follow-up sample was obtained 8-19 months after their surgery. In addition, liver and gallbladder biopsies were collected at time of KPE for comparison to biopsies collected from patients with biliary atresia. The second control group included 22 non-cholestatic infants undergoing surgery due to inguinal hernia. Blood samples from this group were used for comparison between cholestasis and normal liver function in infants. The group was divided into two age groups to also control for age induced changes, one group was aged up to 12 weeks (matching the age of the biliary atresia patients at time of KPE) and the second group was 13 weeks and up

to 10 months of age (matching the age of the biliary atresia patients at follow-up). Blood samples were centrifuged to retrieve the serum fraction and samples were stored at -80°C. Tissue biopsies were snap frozen in liquid nitrogen and stored at -80°C or formalin-fixed prior to paraffin embedding.

Study III: Primary human hepatocytes were isolated from patients undergoing liver resection, from extirpated livers or from donor livers rejected for transplantation. Cells were isolated as described in section 3.3 and were kept in culture for a total of five days. Four different types of experiments were conducted.

1. Dose-response experiments; primary human hepatocytes were treated with 3-20 μM CDCA (Sigma-Aldrich, St. Louis, MO, USA) or 400-1200 pg/mL recombinant FGF19 (R&D system, Minneapolis, MN, USA) for either 6 h (n=10) or 24 h (n=13). CYP7A1 gene expression was measured as described in section 3.4. In addition, gene expression of FGF19 and FGF19 protein in cell medium (section 3.5) was measured in CDCA treated cultures at both time points. Cell medium from five livers at each time point was analyzed for bile acids as described in section 3.6. Cells from three livers treated for 6 h with 10 μM CDCA or 1000 pg/mL FGF19 were analyzed by RNA sequencing as described in section 3.8.
2. Primary human hepatocytes were stimulated to produce endogenous FGF19 (conditioned medium) that was subsequently used for treatment of naïve cells, n=10 (Figure 5). CYP7A1 and FGF19 gene expression was measured (section 3.4). FGF19 protein in cell medium was analyzed (section 3.5). Cell medium from five livers was analyzed for bile acids (section 3.6). Cells from three livers treated with 100 percent conditioned medium or control medium were analyzed by RNA sequencing (section 3.8).
3. Time course experiments; cells were treated with 10 μM CDCA for 10 min up to 6 h (n=3). Gene expression of FGF19 (section 3.4) and FGF19 protein in cell medium (section 3.5) was analyzed.
4. FGF19 expression was knocked down using FGF19 siRNA (n=3). CYP7A1 and FGF19 gene expression was measured (section 3.4). FGF19 protein in cell medium was analyzed in (section 3.5). Cell medium was analyzed for bile acids (section 3.6).

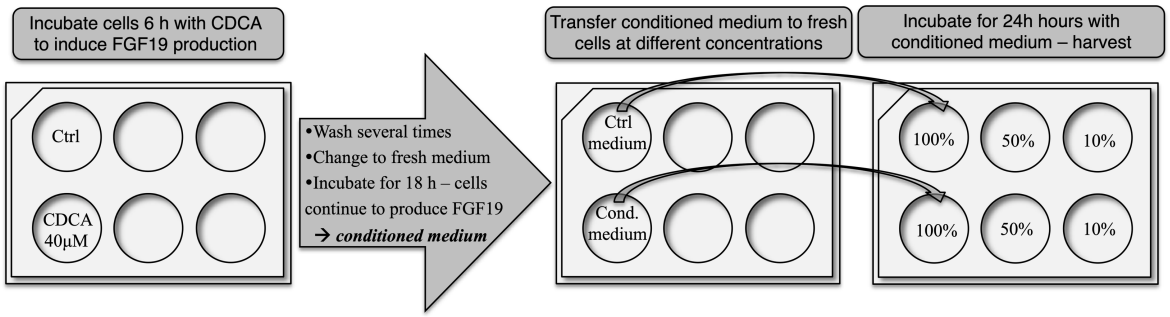

Figure 5. CDCA was used to induce FGF19 production in primary human hepatocytes. After removal of CDCA containing medium and several washes, the cells were left for an additional 18 h and continued to produce FGF19. Medium enriched with FGF19 was then transferred to untreated cells at different concentrations and incubated for 24 h prior to harvesting the cells.

3.3 ISOLATION AND CULTURING OF PRIMARY HUMAN HEPATOCYTES

The isolation procedure followed a three-step perfusion technique as described by Berry and Friend [92] and optimized for primary human hepatocytes by Strom et al. [93]. Briefly, the liver was perfused with buffer containing EGTA – a chelating agent that binds calcium and separates the cells by breaking up desmosomes. After washing out the first solution with buffer, collagenase solution was perfused through the liver to digest the tissue. Cells were cultured for five days on an extracellular matrix in William's E medium supplemented with 20 mM HEPES, 2 mM glutamine, 10 nM insulin, 100 nM dexamethasone, 10 mM gentamicin and 55 nM amphotericin B.

3.4 QUANTIFICATION OF RNA

Total RNA from tissue (Study I and II) and from primary human hepatocytes (Study III) was isolated using TRIzol reagent (Invitrogen, Carlsbad, CA, USA) according to instructions from the manufacturer. cDNA synthesis from 1 μg of RNA was performed using Applied Biosystems' high Capacity cDNA reverse transcription kit (Applied Biosystems, Foster city, CA, USA). Quantification was performed in technical triplicates with TaqMan assays and analyzed on a Step-One Plus instrument. Relative gene expression was calculated against housekeeping genes Cyclophilin A and GAPDH.

3.5 QUANTIFICATION OF PROTEIN

Quantification of FGF19 protein in serum samples (Study I and II) and in cell medium (Study III) and insulin (Study I) was performed using commercial ELISA kits, following the manufacturer's instructions (Human FGF19 Quantikine ELISA kit, R&D system,

Minneapolis, MN, USA and Human insulin ELISA kit, Invitrogen, Carlsbad, CA, USA). All samples were analyzed in technical triplicates.

3.6 QUANTIFICATION OF BILE ACIDS

Total levels of LCA, DCA, CDCA, CA and UDCA in serum (Study I) and in cell medium (Study II) were extracted and analyzed with gas chromatography-mass spectrometry (GC-MS) as first described by Björkhem and Falk [94]. Deuterium-labeled bile acids (D_4-LCA, D_4-DCA, D_2-CDCA and D_4-CA) were used as internal standards. In brief, serum or cell medium was mixed with 2.5 μg internal standard and hydrolyzed with 1 M potassium hydroxide over night. Bile acids were extracted by basic ether extractions followed by acidic ether extractions and neutralized with water. Trimethylsilyl diazomethane was added for methylation and the bile acids were derivatized with a mixture of hexamethyldisilazane and trimethylchlorosilane in pyridine. Quantification of bile acids in the samples was performed with GC-MS (6890 Network GC system/5973 Network mass selective detector), using the MassHunter Workstation software (Agilent Technologies, Santa Clara, CA, USA). All reagents including internal standards were purchased from Sigma-Aldrich (St. Louis, MO, USA).

In Study II, free, taurine and glycine conjugated LCA, DCA, CDCA, CA and UDCA and the bile acid intermediate C4 were analyzed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) in 50 μL serum and 20-50 mg liver tissue following the protocol by Al-Khaifi *et al*. [71]. In short, serum or homogenized liver tissue was mixed with internal standard (10 ng D_7-C4 and 25 ng of each D_4-labeled free- and conjugated bile acid) and acetonitrile. Following centrifugation, the supernatant was transferred and dried with nitrogen and the residue was dissolved in methanol and H_2O. Quantification was performed with a Xevo TO-TX Triple Quadrupole Mass Spectrometer and data analyzed with MassLynx MS Software (Waters Corporation, Milford, MA, USA).

3.7 STAINING OF FORMALIN-FIXED PARAFFIN-EMBEDDED LIVER TISSUE

In Study II, 5 μm sections of formalin-fixed paraffin-embedded liver biopsies were stained by either *in situ* hybridization or immunohistochemistry. The RNA scope 2.0 *in situ* hybridization kit from Advanced Cell Diagnostics (San Francisco, CA, USA) was utilized for visualization of FGF19 RNA in the liver sections, following the manufacturer's instructions. Protein expression of cytokeratin 7 (CK7) was identified by immunohistochemistry. Briefly, methanol with 0,3 % H_2O_2 was used to block endogenous peroxidase and epitope retrieval

was performed using citrate buffer (pH 6) in a pressure cooker. Following blocking of the sections with 2 % BSA, slides were incubated for 1 h with primary antibody against CK7 (Invitrogen, Carlsbad, CA, USA). Anti-mouse HRP-coupled secondary antibody was applied for 20 min at 37^{o}C (Invitrogen, Carlsbad, CA, USA). Both RNA and protein expression were detected with 3,3'-diaminobenzidine (DAB, Advanced Cell Diagnostics, San Francisco, CA, USA). Slides were counterstained with hematoxylin (Merck, Kenilworth, NJ, USA).

3.8 RNA SEQUENCING

Total RNA was extracted as described in section 3.4 above. Prior to sequencing, ribosomal RNA was removed using Ribo-Zero Gold and RNA sequencing libraries were prepared utilizing Illumina TruSeq Stranded RNA Library Prep kit v2 (Illumina, San Diego, CA, USA) according to manufacturer's protocol. Following reverse transcription, quality control of cDNA libraries was determined and normalized with an Agilent Bioanalyzer instrument according to instructions from the manufacturer. A KAPA-SYBR FAST qPCR kit (Roche, Basel, Switzerland) was used to determine library concentrations. Sequencing run was performed with a NextSeq 500/550 High Output v2 kit on a NextSeq 500 (Illumina, San Diego, CA, USA). Following quality control of reads, adaptor sequences were trimmed and low-quality reads as well as reads aligning to ribosomal RNA genes were removed. High-quality reads were aligned to the genome and differential gene expression was analyzed with edgeR v.3.3.3.

4 RESULTS

4.1 SUMMARY OF STUDY I: CIRCULATING FIBROBLAST GROWTH FACTOR 19 IN PORTAL AND SYSTEMIC BLOOD

In Study I, we measured FGF19 concentrations in portal blood and compared it to FGF19 levels in the systemic circulation.

FGF19, bile acids and insulin in portal and systemic blood under fasted conditions

75 patients undergoing liver surgery were enrolled in the study to investigate FGF19 under fasted conditions. FGF19 concentrations did not differ between portal (median 99 pg/mL) and peripheral arterial (median 102 pg/mL, p=0.70) or central venous blood (median 101 pg/mL p=0.85) (Figure 6). Total bile acids and insulin levels were 2-fold higher in portal blood (median 12.7 µM and 58.7 µIU/mL, respectively) compared to peripheral arterial blood (median 6.7 µM, p<0.001 and 25.8 µIU/mL, p<0.001, respectively) and central venous blood (median 6.7 µM, p<0.001 and 25.9 µIU/mL, p<0.001, respectively).

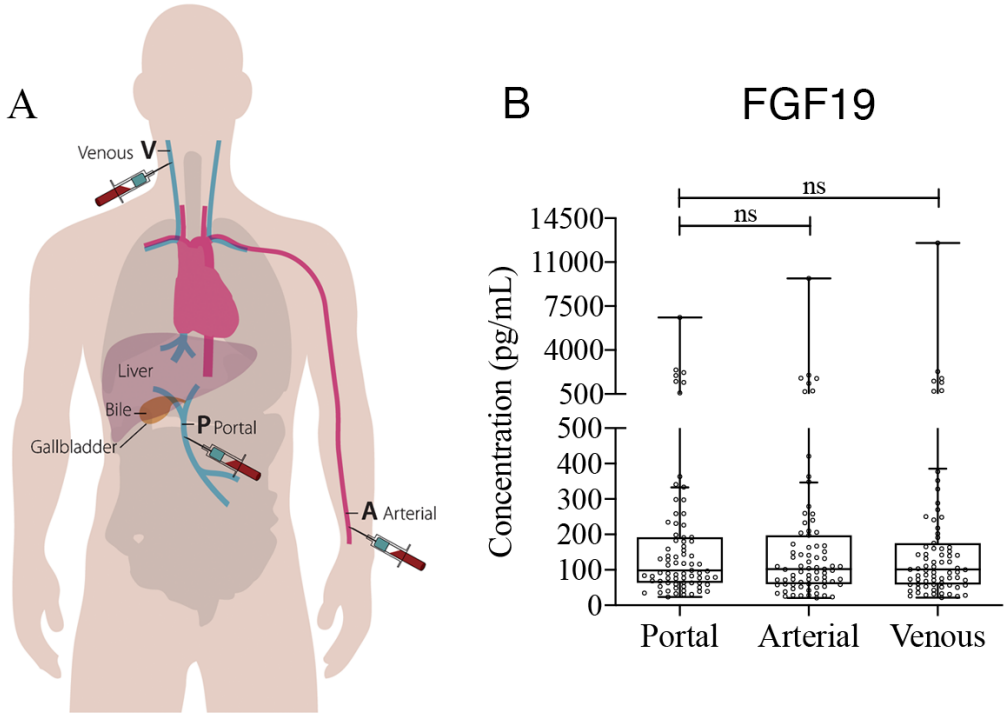

Figure 6. (A) Portal, peripheral arterial and central venous blood was collected from 75 patients undergoing liver surgery. **(B)** FGF19 concentrations in portal blood did not differ from levels in peripheral arterial or central venous blood. Data is presented as median [min-max].

When the study subjects were divided into groups according to diagnosis it was noted that subjects with cholestatic liver disease or cirrhosis did not present a gradient of bile acids over the liver. This most likely reflects a reduced uptake by the liver and increased efflux of bile acids from the liver to the systemic circulation. The patient group with cholestatic liver diseases had median levels of circulating FGF19 that was 4-fold higher than the median levels in donors. There was no significant difference in FGF19 levels between portal and systemic blood in any of the patient groups as shown in Table I (unpublished data).

Table I. Bile acids and FGF19 levels in portal and systemic blood in each patient group, presented as median values [min-max]. Friedman's test with Dunn's multiple comparisons test post-hoc was used to assess differences. Differences were considered significant at $p<0.05$. No difference between peripheral arterial and central venous blood was observed in any of the patient groups for bile acids or FGF19 and the reported p-values are thus comparisons between portal blood and peripheral arterial and central venous blood respectively.

Diagnosis	N (75)	Blood samples [f]	Bile acids (µM)	FGF19 (pg/mL)
Cholestatic liver disease [a]	11	P PA CV	80 [20-265] 61 [18-302] [ns] 59 [18-315] [ns]	298 [49-6600] 280 [53-9700] [ns] 288 [51-9700] [ns]
Cirrhosis [b]	9	P PA CV	34 [10-141] 30 [7-134] [ns] 29 [7-132] [ns]	120 [59-1419] 110 [50-1359] [ns] 100 [49-1419] [ns]
Cancer [c]	40	P PA CV	9.6 [1.5-48.3] 4.9 [1.4-28.3] [p<0.001] 4.6 [1.4-30.0] [p<0.001]	98 [24-1964] 94 [21-1775] [ns] 93 [22-1750] [ns]
Donors [d]	8	P PA CV	4.1 [2.1-9.5] 1.6 [1.0-3.0] [p=0.01] 1.6 [1.0-3.0] [p=0.02]	77 [39-144] 71 [28-141] [ns] 75 [29-142] [ns]
Other diagnosis [e]	7	P PA CV	21.4 [11.4-261] 8.0 [4.7-249] [p=0.01] 8.6 [4.0-249] [p=0.02]	191 [35-2209] 173 [33-2010] [ns] 175 [36-2299] [ns]

a. Primary sclerosing cholangitis (n=5), Progressive familial intrahepatic cholestasis (n=3), Biliary atresia (n=2), Cholestasis unknown cause (n=1) **b.** Alcohol induced cirrhosis (n=6), Secondary biliary cirrhosis (n=1), Alpha-1 antitrypsin deficiency (n=1), Autoimmune hepatitis (n=1) **c.** Colorectal metastasis (n=22), Hepatocellular carcinoma (n=6), Cholangiocarcinoma (n=6), Gallbladder cancer (n=2), Metastasis from Pseudomyxoma peritonei (n=1), Metastasis of Wilms' tumor (n=1), Metastasis of anal cancer (n=1), Intraductal papillary mucinous neoplasm (n=1) **d.** Donor with Familial amyloidotic polyneuropathy (n=4), Living donor (n=3), Brain dead donor (n=1) **e.** Maple syrup urine disease (n=1), Crigler-najjar (n=1), Polycystic liver disease (n=1), Bile duct stricture (n=1), Acute intermittent porphyria (n=1), Acute liver failure (n=2) **f.** Blood samples: P=portal blood, PA=peripheral arterial blood, CV=central venous blood

Relationship between bile acids and FGF19

The relationship between levels of bile acids and FGF19 was investigated and we found a correlation between FGF19 and bile acids in both portal blood ($Rs=0.58$, $p<0.001$) and in systemic blood (peripheral arterial blood $Rs=0.57$, $p<0.001$ and central venous blood $Rs=0.57$, $p<0.001$).

Postprandial increase of bile acids and FGF19 in systemic blood

In the second part of the study, we investigated if the postprandial surge of FGF19 could give rise to a concentration gradient of FGF19. Bile flow to the intestine under fasted conditions is reduced [95], which could affect FGF19 gene expression in the intestine and consequently circulating concentrations. Analysis of systemic blood samples (peripheral arterial and/or central venous blood) at the two time-points, showed that bile acids increased 2-fold in donor 1, 1.6-fold in donor 2 and 1.3-fold in donor 3 between baseline and two hours after administration of a high-energy nutrient drink. FGF19 increased 1.5-fold in donor 1, was unchanged in donor 2 and increased 2-fold in donor 3.

Postprandial concentrations of bile acids and FGF19 in portal blood

Bile acids two hours after nutrient intake were 5-fold higher in portal blood than the concentration in systemic blood collected at the same time-point in donor 1 and 3, and 3-fold higher in donor 2. The postprandial FGF19 concentration in portal blood did not differ from systemic blood.

4.2 SUMMARY OF STUDY II: REGULATION OF BILE ACID METABOLISM IN BILIARY ATRESIA: REDUCTION OF FGF19 BY KASAI PORTOENTEROSTOMY AND POSSIBLE RELATION TO EARLY OUTCOME

In Study II, we aimed to investigate expression site and circulating levels of FGF19 in children with biliary atresia at time of KPE and possible changes at six-months follow-up.

Circulating FGF19, bile acids and C4 in children with biliary atresia at time of KPE and at six-months follow-up

Circulating FGF19 levels were four times higher in portal and peripheral blood at time of KPE (329 pg/mL and 320 pg/mL respectively) than at the time of follow-up (79 pg/mL, p=0.01 and p=0.002) in patients with biliary atresia. No difference was found in FGF19 concentrations between portal and systemic blood at KPE (p>0.99). The levels of FGF19 in blood at time of KPE correlated to gene expression of FGF19 in liver (Rs=0.86, p<0.001) and it was concluded that the circulating levels most likely originated from the liver in this condition. Furthermore, staining of FGF19 RNA in liver sections revealed expression in hepatocytes but not in other cell types, such as cholangiocytes (Figure 7).

The circulating levels of FGF19 in biliary atresia at time of KPE were significantly higher than circulating levels in non-cholestatic infants (median level in the older group: 97 pg/mL, p=0.04 and median level in the younger group: 105 pg/mL, p=0.04). On the other hand, circulating FGF19 in biliary atresia at follow-up did not differ from the levels in non-cholestatic infants (p>0.99 for both age groups).

Bile acids remained unchanged between KPE and follow-up (411 and 457 μM respectively, p=0.76) and were 100-fold and 400-fold higher in biliary atresia compared to the younger and older age group of non-cholestatic infants respectively. C4 levels increased between KPE (1.2 ng/mL) and follow-up (2.1 ng/mL, p=0.01).

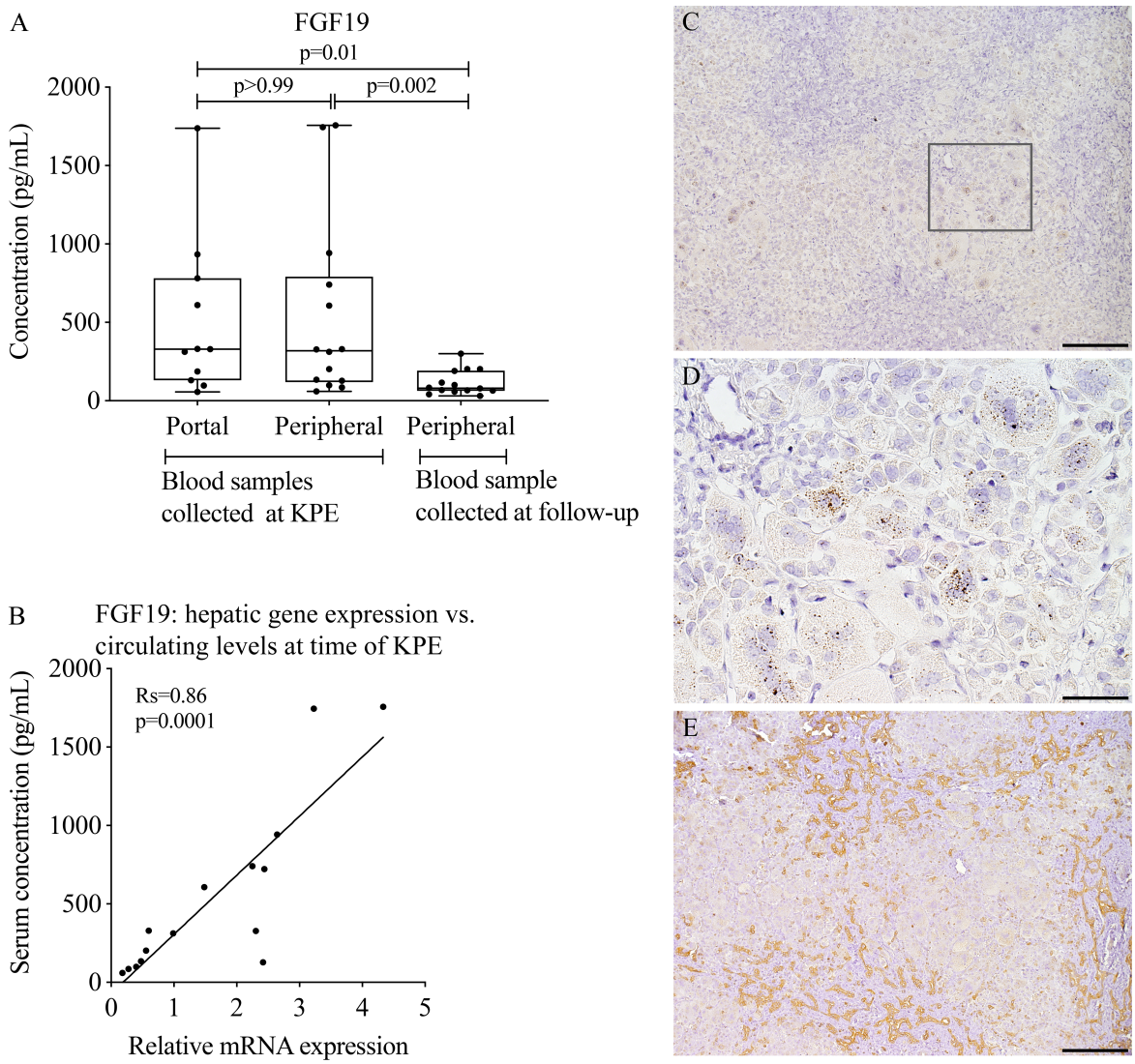

Figure 7. (A) Circulating FGF19 levels at time of KPE were four times higher than the circulating levels at six-months follow-up. Data is presented as median [min-max]. **(B)** Circulating levels of FGF19 correlated to gene expression in liver at time of KPE. **(C-E)** Staining of FGF19 at 10x magnification (C) and 40x magnification (D) and CK7 at 10x magnification (E). (D) Represents the grey square in (C). (C) and (E) are consecutive sections from the same liver specimen. FGF19 was found in hepatocytes but not in cholangiocytes.

Decline in circulating FGF19 levels following KPE was related to normalization of bilirubin and survival of native liver at 2 years of age.

At the age of 2 years, seven of the children diagnosed with biliary atresia had been transplanted while eight children had survived with their native liver. At six-months follow-up, the children with survival of their native liver had normalized bilirubin (142 µM at KPE and 11 µM at follow-up, p=0.008) and the median levels of circulating FGF19 decreased from 257 pg/mL to 71 pg/mL, p=0.008. Total bile acids remained unchanged (KPE; 358 µM and follow-up 302 µM, p=0.8) but conjugated CDCA was significantly reduced (121 µM to

46 μM, p=0.04), while levels of free bile acids increased (CA; 0.03 μM to 0.08 μM p=0.02, CDCA; 0.02 μM to 0.3 μM p=0.02 and UDCA; 1 μM to 18 μM p=0.008). C4 remained unchanged (1.3 ng/mL at KPE and 2.4 ng/mL at follow-up, p=0.25). The children who underwent transplantation before the age of 2 years had no significant changes in bilirubin (KPE; 186 μM and follow-up; 181 μM, p=0.31) or total bile acids (KPE; 500 μM and follow-up; 502 μM, p=0.8). C4 and free CDCA increased (C4; 1.2 ng/mL to 2.1 ng/mL, p=0.02 and CDCA; 0.03 μM to 0.1 μM, p=0.03). No significant reduction in FGF19 was observed (KPE; 329 pg/mL and follow-up; 194 pg/mL, p=0.15).

Gene expression and bile acids in liver biopsies

Hepatic gene expression of factors involved in synthesis, regulation and transport of bile acids were examined. Gene expression of key enzymes in bile acid synthesis CYP7A1, CYP7B1 and CYP27A1 were lower in biliary atresia when compared to expression in patients with choledochal malformation (p=0.05, p=0.025 and p=0.019 respectively) while CYP8B1 did not differ (p=0.098). The bile acid uptake transporter NTCP displayed a reduced expression (p=0.0015), while sinusoidal efflux transporters MRP4 and OSTβ were increased (p=0.0014 and p<0.001). Expression of OSTα and MRP3 did not differ between the groups (p=0.67 and p=0.098) and neither did the expression of the canalicular export pump BSEP (p=0.55). FGF19 was significantly higher expressed in biliary atresia (p=0.008) while expression of its cognate receptor FGFR4 and co-factor βKlotho did not differ (p=0.35 and p=0.55). Gene expression of SHP did not differ between biliary atresia and choledochal malformation (p=0.20) while FXR expression in biliary atresia was lower than in choledochal malformation (p<0.001). The median level of total bile acids in liver tissue was 2.9 μmol/g protein.

FGF19 gene expression in liver versus gallbladder in biliary atresia and choledochal malformation

One interesting finding was the reversed relationship of FGF19 gene expression in gallbladder and liver in biliary atresia and children with choledochal malformations (unpublished data). Gallbladder remnants were collected from children with biliary atresia and a gallbladder biopsy was collected from the choledochal patients. FGF19 gene expression level was 340 times higher in liver than gallbladder in children with biliary atresia. On the other hand, FGF19 gene expression in choledochal malformation was 76 times higher in gallbladder than in liver (Figure 8).

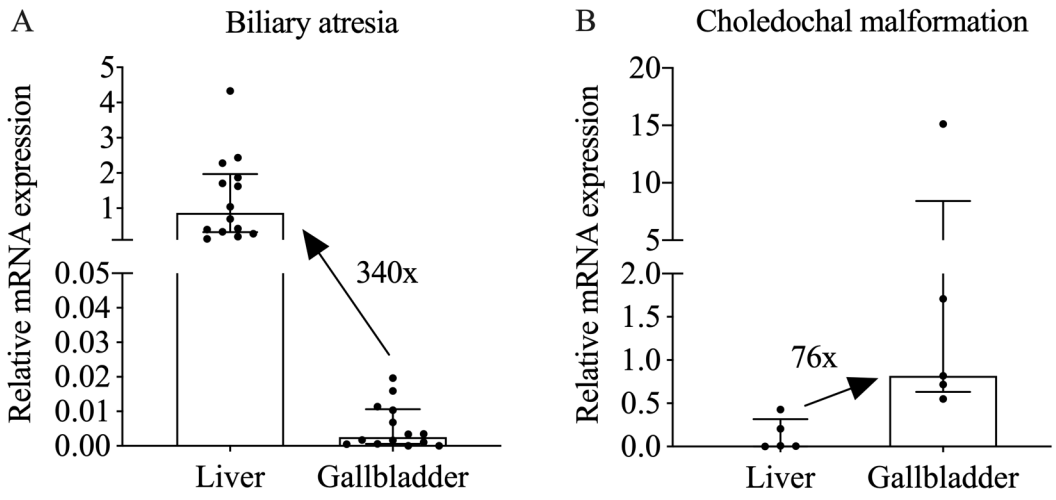

Figure 8. (A) FGF19 gene expression in liver was 340x higher than gallbladder in biliary atresia while **(B)** the gene expression in gallbladder was 76x higher in gallbladder than liver in children with choledochal malformation. Data is presented as median with interquartile range.

4.3 SUMMARY OF STUDY III: CHENODEOXYCHOLIC ACID MODULATES BILE ACID SYNTHESIS INDEPENDENT OF FIBROBLAST GROWTH FACTOR 19 IN PRIMARY HUMAN HEPATOCYTES

Here, we studied the effects of FGF19 and CDCA on bile acid synthesis in primary human hepatocytes.

The effect of CDCA and recombinant FGF19 treatment on primary human hepatocytes on CYP7A1 gene expression and CA formation

CYP7A1 gene expression was significantly reduced following treatment with CDCA for 6 h (10 μM $p=0.002$, 15 μM $p<0.001$ and 20 μM $p<0.001$) and 24 h (10 μM $p<0.001$, 15 μM $p<0.001$ and 20 μM $p<0.001$). CA levels in cell medium were lower than in control cultures following 24 h treatment with CDCA at concentrations of 15 μM ($p=0.04$) and 20 μM ($p=0.02$). On the other hand, recombinant FGF19 (400-1200 pg/mL) had no significant effect on CYP7A1 gene expression or CA formation at either concentration or time point.

CDCA up regulated FGF19 gene expression in primary human hepatocytes

FGF19 gene expression in primary human hepatocytes increased in a dose-dependent manner following treatment with 3-20 μM CDCA. In accordance, FGF19 protein secretion into cell medium also appeared and increased in a dose-dependent manner to CDCA. A time-course experiment with 10 μM CDCA revealed a fast induction of FGF19 gene expression occurring 1-1.5 h following treatment with subsequent protein secretion to the cell medium with a 2 h delay.

The effect of cell medium enriched with endogenously produced FGF19 on CYP7A1 gene expression and CA formation in primary human hepatocytes

The low response of primary human hepatocytes to recombinant FGF19 was perplexing. It has been suggested that recombinant FGF19 has decreased biological activity [96] and we therefore designed an experiment to investigate effects of endogenously produced FGF19 on bile acid synthesis. Cells were treated with medium containing FGF19 produced by primary human hepatocytes as described in Figure 5. The median level of FGF19 in the conditioned medium was 310 pg/mL (Figure 9A). CYP7A1 gene expression was lower in cells treated with conditioned medium compared to control cultures, Figure 9B ($p=0.05$), while CA formation remained unchanged. The conditioned medium contained CDCA levels up to 2 μM.

A

FGF19 (pg/mL) in cell medium		
	Control medium	Conditioned medium
10%	23 [8-56]	38 [14-77]
50%	22 [5-74]	140 [53-327]
100%	25 [6-90]	310 [113-541]

B

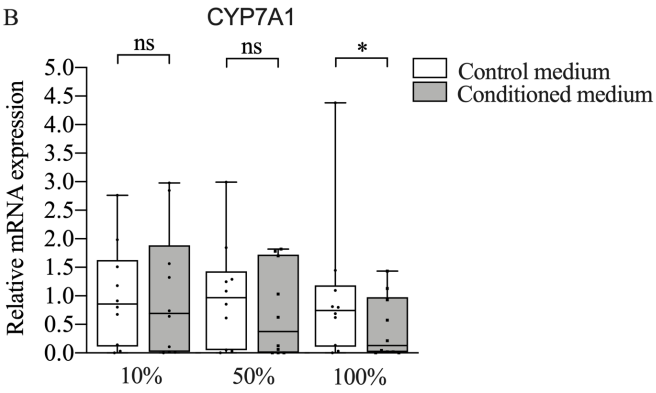

Figure 9. (A) FGF19 concentration in control and conditioned cell medium. **(B)** CYP7A1 gene expression after treatment with medium containing endogenously produced FGF19. Data is presented as median [min-max].

RNA sequencing of primary human hepatocytes treated with CDCA, recombinant FGF19 or medium enriched with endogenously produced FGF19

To further discern possible molecular mechanisms of CDCA and FGF19, a global gene expression analysis was performed on cells treated with 10 μM CDCA or 1000 pg/mL of recombinant FGF19 and on cells treated with conditioned medium. CDCA treatment altered the expression of 627 genes. Among them were genes involved in bile acid metabolism, *e.g.* SHP (up regulated 2.7-fold), FGF19 (up regulated 22.7-fold) and CYP7A1 (down regulated 26.4-fold). Pathway analyses showed that CDCA affected genes involved in lipid and bile acid transport and secretion (*e.g.* BSEP, and OSTα/OSTβ). Recombinant FGF19 altered the expression of 476 genes but CYP7A1 was not among the differentially expressed genes. Furthermore, pathway analyses did not confirm any pathway that was significantly altered upon treatment with recombinant FGF19. Cells treated with medium containing endogenously produced FGF19 altered the expression of 304 genes and CYP7A1 was among the differentially expressed genes (down regulated 6.1-fold). FGF19 and OSTβ were up regulated 5.0 and 2.9-fold respectively, in cells treated with conditioned medium. These genes are associated with bile acid activation of FXR and could result from CDCA in the conditioned medium. Pathway analyses did not reveal any pathways that were significantly altered by conditioned medium with endogenous FGF19.

CYP7A1 gene expression in primary human hepatocytes in presence of CDCA following siRNA-mediated knockdown of FGF19

FGF19 was induced rapidly by CDCA in primary human hepatocytes and conditioned medium with endogenous FGF19 down regulated CYP7A1, which could imply a possible

autocrine function of FGF19 in regulation of bile acid synthesis. However, the contribution of FGF19 relative to other factors (*e.g.* bile acids produced by the cells) warranted further evaluation of a possible autocrine pathway for down regulation of CYP7A1 by FGF19. A knockdown experiment of FGF19 was therefore performed. FGF19 gene expression and protein secretion was efficiently reduced following transfection with siRNA against FGF19 and was not induced by CDCA administration to the cells (Figure 10 A-B). CYP7A1 gene expression was 43-fold lower in cells following treatment with 10 µM CDCA compared to controls, irrespectively of siRNA-mediated depletion of FGF19 (Figure 10 C). Levels of CA in cell supernatants remained unchanged.

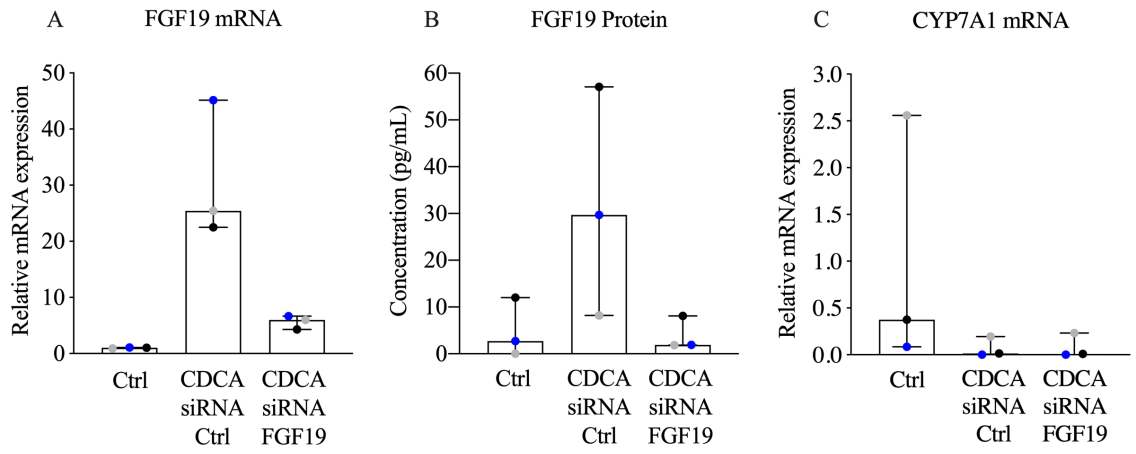

Figure 10. (A) Gene expression of FGF19 **(B)** Protein concentration of FGF19 in cell medium **(C)** CYP7A1 gene expression.

5 DISCUSSION AND FUTURE PERSPECTIVES

Fundamental species differences in bile acid metabolism necessitate studies in humans and human cell models to fully understand molecular and physiological mechanisms that are operating to maintain bile acid homeostasis in man. Human studies are of importance for discerning underlying causes of diseases affecting bile acid metabolism and for finding new therapeutic targets in such conditions. The studies included in this thesis are focused on using human models to further understand the role of FGF19 in human bile acid metabolism in health and cholestatic liver disease.

Physiological levels of circulating FGF19

A key function of the liver is biotransformation of both exogenous and endogenous compounds to enable excretion through bile or urine [1,97]. First pass metabolism by the liver can result in significant differences in concentration and properties of substances in portal blood compared to the systemic circulation. This has to be accounted for when *e.g.* developing orally ingestible drugs [97].

Bile acids are examples of endogenous compounds that are readily absorbed and transformed by the liver, as described in the introduction. Insulin is another example of an endogenous peptide that similar to bile acids displays a gradient over the liver, *i.e.* with higher concentrations in portal blood than systemic blood [31,98,99]. For the set up and interpretation of experiments and for distinguishing physiological levels from abnormal levels it is therefore of importance to know if there are changes in concentration or properties of compounds, such as FGF19, after transit through the liver. However, measuring substances in portal blood is complicated since the portal vein is situated deep inside the abdomen. Animal models may not be useful due to species differences and measuring the protein of the FGF19 mouse ortholog Fgf15 in blood samples has also proven difficult [66,100,101]. It was not known until recently if the circulating levels of FGF19 found in peripheral blood reflect portal concentrations [72,81,102].

We demonstrated in Study I that while bile acids were higher in portal than systemic blood under fasted conditions, the FGF19 levels were not significantly different (Figure 6). We furthermore observed that though there was a large interindividual difference of circulating FGF19, there was no gradient over the liver of FGF19 in none of the patient groups (Study I, Table 1 and Study II, Figure 7). Koelfat et al. [102] reported similar results in a group of fasted patients undergoing liver surgery. Though they did observe a small but significant

elevation in portal blood compared to arterial (but not venous) blood they concluded that the difference was too small to indicate a hepatic clearance.

Under fasted conditions, a pattern of electromechanical activity in the intestine called the migratory motor complex stimulates bile secretion to the intestine. However, intestinal bile acid levels are reduced in a non-fed state and that could consequently affect expression and subsequently the concentration of FGF19 in blood [95,103]. Postprandial changes in bile secretion to the intestine and the resulting increase in circulating FGF19 thus prompted us to investigate portal levels of FGF19 under food-stimulated conditions to confirm our observations under non-fed conditions. As expected, both bile acids and FGF19 increased following food intake. Similar to what was observed under fasted condition, no differences were seen in FGF19 levels between portal and systemic blood postprandially. Bile acid concentrations were up to five times higher in portal blood than systemic blood, in agreement with previous studies [31]. FGF19 in the systemic circulation reflect portal blood concentrations and circulating levels of FGF19 can thus be interpreted from systemic blood. Knowledge of the physiological levels of circulating FGF19 should be of importance for future studies to evaluate the relevance of FGF19 in the liver and in bile acid metabolism both under physiological conditions and in conditions where levels are elevated [56,64–66,69,80].

The modified response of FGF19 in cholestatic liver disease

Cholestasis results in a number of counteractive metabolic responses and much of the adaptive response of the liver aim at decreasing intracellular levels of bile acids, which is crucial to reduce bile acid induced toxicity and cell damage [14,69,78,79]. In Study II, accumulation of bile acids in liver tissue of patients with biliary atresia was found, consistent with published data [104]. In accordance, several adaptions that have been attributed to increased hepatic levels of bile acids and subsequent activation of FXR were observed [69,79]. Bile acid synthesis was down regulated, revealed by decreased CYP7A1 gene expression and low levels of circulating C4. In addition, a reduced expression of the main uptake transporter NTCP in combination with an increased expression of sinusoidal export transporters was found. Altogether this indicates that the hepatocytes are trying to increase efflux of bile acids, while inhibiting uptake and *de novo* synthesis to decrease bile acid induced toxicity [78,79].

In agreement with studies reporting modifications of FGF19 in cholestasis [69,80], we observed approximately four times higher levels of circulating FGF19 in patients with

cholestatic liver diseases compared to donors in Study I (Table I). The increased levels and altered gene expression of FGF19 in cholestasis has been speculated to be part of the adaptive response by the liver induced by high intracellular levels of bile acids that aim to inhibit bile acid synthesis [69,79,80]. In contrast, the BDL mouse model, where bile flow from the liver has been restricted by ligation of the bile duct, does not express Fgf15 in the liver and the mouse model display increased expression of Cyp7a1 and an increased bile acid pool, further demonstrating the species differences between human and mouse [66,69,81].

In Study II, the levels of circulating FGF19 at time of KPE was elevated in patients with biliary atresia and an increased gene expression of FGF19 in the liver was found located to the hepatocytes. Moreover, circulating levels and gene expression in liver showed a positive correlation leading us to conclude that the elevated serum levels of FGF19 most likely originated from the hepatocytes (Figure 7). The high hepatic gene expression of FGF19 is likely an effect of increased hepatic bile acid levels [56,66,70]. Interestingly, SHP was not increased, which is in agreement with other studies [69,79]. The hepatic pathway for suppression of bile acid synthesis thus appears to be diminished in prolonged cholestasis, which implicates that FGF19 could have an important function in down regulating bile acid synthesis in this situation. There are however no direct measurements of how FGF19 impacts bile acid synthesis during cholestasis. Further studies are needed on FGF19 in cholestasis to fully understand the extent of its involvement in the adaptive response of the cholestatic liver.

FGF19 as a prognostic and therapeutic target in cholestatic liver disorders

The compensatory mechanisms discussed here are unable to fully prevent long-term hepatic damage in prolonged cholestasis. Currently, ongoing studies are investigating therapeutic agents targeting or enhancing regulatory factors in bile acid metabolism to decrease damage induced by bile acids in cholestasis [41,78,105–107]. Given its role as a regulator of bile acid synthesis, FGF19 has been proposed to be a candidate for a therapeutic target in cholestasis [108–110]. Both FGF19 and FGFR4 are however associated with development and progress of hepatocellular carcinoma (HCC) [111,112]. To circumvent this problem, a nontumorigenic variant of FGF19 has been engineered called NGM282 or M70. The M70 protein still display biological activity and suppress bile acid synthesis [108,109]. A recent phase 2 study on patients with primary biliary cholangitis (PBC) has furthermore shown positive results with improved liver status following treatment with M70 [107]. The beneficial effects of administrating exogenous FGF19 in severe cholestasis, such as biliary atresia, for the purpose of down regulating bile acid synthesis is however arguable since bile acid synthesis is already suppressed to its minimum. Nonetheless, it has been speculated that administrating the

nontumorigenic FGF19 could reduce carcinogenic effects of high levels of endogenous FGF19 in long-term cholestasis by competing over binding to FGFR4 [110]. In addition to clinical implications for a drug, a possible prognostic value of FGF19 in cholestasis has been discussed [80]. Taken together, FGF19 remains as an interesting marker for prognosis and target for clinical management of cholestatic liver disease.

In Study II, we found a decrease of circulating FGF19 in patients with biliary atresia between KPE and follow-up (Figure 7) that could not be explained by neither the surgical procedure [113] nor age-related changes [114]. Given that circulating FGF19 was proportional to the hepatic gene expression, we hypothesized that the decreased levels of FGF19 could reflect a loss of hepatocytes being replaced by fibrotic tissue and thereby indicating a less favorable outcome of surgery. To further investigate a possible relation in changes of circulating FGF19 to clinical outcome of the surgery we divided the children based on survival of native liver at the age of 2 years. We found that the reduction of circulating FGF19 was less pronounced in the children who underwent transplantation before the age of 2 years, while a significant reduction was observed in the children with survival of their native liver at the age of 2 years. The decrease of FGF19 in this group coincided with normalization of markers that would suggest a restored bile flow [85,115], namely a reduction of bilirubin and conjugated CDCA. Altogether, this may indicate that reduced levels of FGF19 indicate a restoration of the enterohepatic circulation post-surgery. A decrease of intrahepatic bile acids as a result of bile flow restoration may decrease hepatic gene expression of FGF19 and consequently circulating levels. We observed a decrease, but not normalization, of bilirubin levels in some of the children who underwent liver transplantation before the age of 2. This decline may indicate a partially restored bile flow that however was not sufficient in stopping the progress of the disease. Nonetheless, this may explain why FGF19 was decreased also in some of the individuals in the transplanted group at the time of follow-up. To better understand the molecular mechanisms behind the decrease of FGF19 would be of interest, but would require repeated liver biopsies. The possibility of FGF19 as an indicator of clinical improvement post KPE is interesting and also warrants further studies. To measure FGF19 at more than one time-point post KPE may provide useful information: it would be interesting to examine how fast FGF19 declines after the surgery and if the differences between the survival group and the transplanted group are more or less distinguished at different time points.

In vitro studies of FGF19's role in regulation of bile acid synthesis

Current understanding of FGF19 in humans is to a large extent based on studies utilizing animal models or cell systems where FGF19 has been administrated at considerably higher concentrations than what is found in the human blood circulation [56,64,65,116]. The physiological relevance of these results is difficult to ascertain, however it cannot be excluded that the recombinant protein has lower biological activity than the endogenous protein. In Study III, we found a weak response in primary human hepatocytes to physiological levels of recombinant FGF19. Notably, FGFR4 and βKlotho were stably expressed by the hepatocytes. In contrast, low concentrations of CDCA had the expected effect on bile acid synthesis by efficiently inhibiting CYP7A1 and, as visualized by the global analysis, also affected other target genes associated with bile acid activation of FXR [34–36]. A study by Kong and Guo [96] suggested that Fgf15 forms inclusion bodies, *i.e.* aggregates of the protein, when it is expressed in *Escherichia coli*. These aggregates do not re-fold properly *in vitro* following isolation and purification, which may render the recombinant protein less biological active. This encouraged us to carry out experiments with endogenously produced FGF19.

The conditioned medium contained endogenously produced FGF19 at levels that would be considered physiological postprandially (Figure 9A) [70]. Moreover, primary human hepatocytes that were treated with conditioned medium had a decreased gene expression of CYP7A1 (Figure 9B). However, as the endogenously produced FGF19 was not purified and the conditioned medium contained CDCA further validation of the impact of endogenously produced FGF19 and CDCA respectively is needed. *In vitro* experiments ahead may for example include conditions where the hepatic pathway of SHP has been silenced to gain further insight to the impact of endogenously produced FGF19 on bile acid synthesis.

An autocrine function of FGF19 in regulation of bile acid synthesis?

Time-course experiments in Study III showed that FGF19 was efficiently induced in primary human hepatocytes by physiological levels of CDCA, with gene expression starting to increase after one hour followed by protein secretion after two hours. This is within the time frame of the postprandial peak of FGF19 observed in blood following the peak of bile acids [70,72]. Whether hepatic FGF19 is induced by bile acids *in vivo* in subjects not affected by cholestasis, and contributes to circulating FGF19 is unclear from this set-up however, since much of the interplay of other factors has not been accounted for. CDCA would normally be mixed with other bile acids that might compete for binding to FXR and that are less efficient to activate it [10]. Nevertheless, we designed an experiment to evaluate if bile acid synthesis

is subjected to an autocrine regulation by endogenously produced FGF19 in the hepatocyte. Knockdown of FGF19 had no significant effect on CDCA mediated down regulation of CYP7A1; the expression was reduced 43-fold compared to control, presumably via the FXR-SHP-pathway [11]. Therefore, it is unlikely that an autocrine signaling of FGF19 has any major role in the negative feedback regulation of bile acid synthesis under normal conditions. Hepatic FGF19 may however, as discussed above, become of regulatory importance in conditions where bile acid metabolism is dysregulated, *e.g.* in cholestasis [69,80,81].

6 CONCLUSIONS

FGF19 has been extensively studied in the past twenty years and is now considered a key player in bile acid homeostasis. In our studies we have determined the levels of circulating FGF19 and how circulating levels and the site of origin changes during cholestatic diseases.

The findings of the studies included in this thesis can be summarized as follows:

Levels of circulating FGF19 in systemic blood reflected the levels in portal blood and this association prevailed in both the fasted and postprandial state of nutrition in both healthy subjects and patients with different types of liver diseases (**Study I** and **II**).

Elevated circulating FGF19 in cholestatic infants originated from the liver. When cholestasis was resolved, FGF19 decreased. This indicates that circulating FGF19 levels could be a possible marker for the progression of cholestatic liver disease (**Study II**).

Physiological levels of recombinant FGF19 had a weak effect on bile acid synthesis in primary human hepatocytes. Conditioned medium containing endogenously produced FGF19 down regulated CYP7A1 gene expression, however further validation is needed to assess the impact of FGF19 in bile acid synthesis from this experiment (**Study III**).

CDCA rapidly induced FGF19 in primary human hepatocytes and at physiological concentrations implying a possible autocrine signaling of FGF19. However, bile acid synthesis was efficiently down regulated by CDCA independently of hepatic FGF19 expression, suggesting that the contribution of hepatic FGF19 in regulation of bile acid synthesis is negligible in the healthy liver (**Study III**).

7 ACKNOWLEDGEMENTS

I would sincerely like to thank everyone who have been involved and contributed to this thesis;

My supervisor, Ewa Ellis, for taking me in as a student a long time ago, for introducing me to the world of bile acids and primary human hepatocytes and for letting me stay on as a PhD-student in your lab. Your talent for thinking outside the box never stops surprising me and your enthusiasm and optimism have encouraged me to not give up even when things sometimes have seemed close to impossible.

My co-supervisors;
Greg Nowak, for all your help setting up and carry out the studies in this thesis. You always seem to have an amazing way of looking at things from the bright side.

Björn Fischler, for sharing your expertise on pediatric liver diseases, for always being a calm and reasonable voice in meetings and discussions and for your invaluable help in setting up and carry out our study on biliary atresia.

Professor Bo-Göran Ericzon, for always asking the interesting questions that have led to sometimes unexpected answers and new ideas.

My mentor Agneta Mode, for introducing me to the Liver cell lab, for sharing your vast knowledge of science and for tirelessly reading and helping me improve the scientific content of my work. Thank you also for asking the question that eventually led us to the first study in this thesis!

To my former and present colleagues and friends in the Liver cell lab;
Helén Zemack, thank you for everything you have taught me in the lab including how to best drive with liquid nitrogen in the car! I am so grateful for all your help in my projects. You are truly one of the kindest people I know.
Lisa-Mari Mörk, Ahmad Karadagi, Makiko Kumagai-Braesch and Lena Berglin; even though we do not all share the same office anymore I'm happy that we still manage to get together for lunches and fikas and for all the scientific and non-scientific talks over the years.
Meng Li, Masaaki Watanabe, Davide Gnocchi, Anna Domogatskaya, Martin Dulac, Kazuaki Tokodai, Staffan Thorén, Janine Attevall and all the students that have passed through the lab; it has been great to share the lab and office with you.

To everyone in the Transplantation unit; Carl Jorns, Gunnar Söderdahl, Ming Yao, Rafael Dlugosz, Henrik Gertzen, Antonio Romano, Torbjörn Lundgren, Johan Nordström and Helena Genberg for taking time out of your work to collect samples for our studies. Thank you also to everyone in the OFO group and especially to Maria Söderström, Ann-Christin Croon and Øystein Jynge for all your help with my first study. Sanna Sörman, Åsa Catapano and Karin Heumann, thank you for administrative assistance during my thesis.

Jan Svensson and Markus Almström, thank you for great collaborations on our study on biliary atresia, your involvement in this study has been invaluable.

To Lena Hallberg and Elin Öst for making sure I got my follow-up samples on time.

To everyone in the Division of Surgery and in particular Bengt Isaksson, Ernesto Sparrelid, Stefan Gilg, Cecilia Strömberg, Ann-Charlotte Elkan, Ioanna Apostolopoulou and Berit Sunde for nice collaborations on the studies in this thesis and those studies that are not included here.

To Professor Stephen Strom and Roberto Gramignoli for all your help during isolations and for sharing your vast knowledge on primary human hepatocytes. Thank you also to all the former and present members of the Strom group for nice chats in the corridors, exchanging reagents whenever we run out and for sharing knowledge on various methods.

Anita Lövgren Sandblom, for sharing your knowledge on analyzing bile acids, helping me set up my methods and for always coming to my rescue when something goes wrong.

Hanns-Ulrich Marshall, for feedback on our manuscripts and suggestions for how to further strengthen the scientific quality of our studies.

Professor Bo Angelin, Professor Mats Rudling and Ingela Arvidsson, for providing your expertise on bile acids and science, and for supporting the work that we have been doing.

Noémi Van Hul, for expert help and advice on stainings and histology and for your positive energy whenever you are in the lab.

Claudia Kutter and Jonas Nørskov Søndergaard, for good collaborations on our third study.

Professor Christopher Liddle, for inviting me to your lab at The Westmead Institute and Sally Coulter, for taking care of me during my visit in Sydney and for teaching me the machine and analysis. I truly had a great time.

To my family and friends for their endless support throughout the years, in particular;
Ann-Britt, Thomas and Marcus
Anne-Lie, Eila, Bengt and Andreas
My aunt Yvonne

My dad, thank you for always trusting and supporting my decisions, for always being there for me and for never telling me what to do.

Martin, for your constant support and for listening to all my talks about bile acids, livers and FGF19. By now I'm sure you know this subject as well as I do. Thank you for being my constant reminder of a more important world outside of work.

If I have forgotten someone, I sincerely apologize.

8 REFERENCES

1. McCuskey R. Anatomy of the Liver. In: Zakim and Boyer's Hepatology: A Textbook of Liver diseases. Saunders; 2012:3-19.

2. Ishibashi H, Nakamura M, Komori A, Migita K, Shimoda S. Liver architecture, cell function, and disease. *Semin Immunopathol* 2009; **31**: 399–409.

3. Jungermann K, Kietzman T. Zonation of Parenchymal and Nonparenchymal Metabolism in Liver. *Annu Rev Nutr* 1996; **16**: 179–203.

4. Tabibian JH, Masyuk AI, Masyuk TV, O'Hara SP, LaRusso NF. Physiology of cholangiocytes. *Compr Physiol* 2013; **3**: 541–65.

5. Keitel V, Häussinger D. Role of TGR5 (GPBAR1) in Liver Disease. *Semin Liver Dis* 2018; **38**: 333–9.

6. Makishima M, Okamoto AY, Repa JJ, *et al.* Identification of a Nuclear Receptor for Bile Acids. *Science* 1999; **284**: 1362–5.

7. Wang H, Chen J, Hollister K, Sowers L, Forman B. Endogenous bile acids are ligands for the nuclear receptor FXR/BAR. *Mol Cell* 1999; **3**: 543–53.

8. Sinal C, Tohkin M, Miyata M, Ward J, Lambert G, Gonzalez F. Targeted Disruption of the Nuclear Receptor FXR/BAR Impairs Bile Acid and Lipid Homeostasis. *Cell* 2000; **102**: 731–44.

9. Kok T, Hulzebos CV, Wolters H, *et al.* Enterohepatic Circulation of Bile Salts in Farnesoid X Receptor-deficient Mice. Efficient Intestinal Bile Salt Absorption in the Absence of Ileal Bile Acid-Binding Protein. *J Bioll Chem* 2003; **278**: 41930–7.

10. Ellis E, Axelsson M, Abrahamsson A, *et al.* Feedback regulation of bile acid synthesis in primary human hepatocytes: evidence that CDCA is the strongest inhibitor. *Hepatology* 2003; **38**: 930–8.

11. Goodwin B, Jones SA, Price RR, *et al.* A regulatory cascade of the nuclear receptors FXR, SHP-1, and LRH-1 represses bile acid biosynthesis. *Mol Cell* 2000; **6**: 517–26.

12. Laarschot L, Jansen P, Schaap F, Damink S. The role of bile salts in liver regeneration. *Hepatol Int* 2016; **10**: 733–40.

13. Chiang J. Bile Acid Metabolism and Signaling. *Compr Physiol* 2013; **3**: 1191–212.

14. Li T, Apte U. Bile Acid Metabolism and Signaling in Cholestasis, Inflammation, and Cancer. *Adv Pharmacol* 2015; **74**: 263–302.

15. Russell D. The enzymes, regulation, and genetics of bile acid synthesis. *Annu Rev Biochem* 2003; **72**: 137–74.

16. Li T, Chiang J. Bile Acid Signaling in Liver Metabolism and Diseases. *J Lipids* 2011; **2012**: 1-9.

17. Axelson M, Ellis E, Mörk B, *et al.* Bile acid synthesis in cultured human hepatocytes: support for an alternative biosynthetic pathway to cholic acid. *Hepatology* 2000; **31**: 1305–12.
18. Ellis E, Goodwin B, Abrahamsson A, *et al.* Bile acid synthesis in primary cultures of rat and human hepatocytes. *Hepatology* 1998; **27**: 615–20.
19. Norlin M, Wikvall K. Enzymes in the Conversion of Cholesterol into Bile Acids. *Curr Mol Med* 2007; **7**: 199–218.
20. Einarsson K, Angelin B. Gallsyrametabolism vid leversjukdom. In: Hepatologi 1. Lunds offset AB; 1988:51–68.
21. Falany CN, Johnson MR, Barnes S, Diasio RB. Glycine and Taurine Conjugation of Bile Acids by a Single Enzyme. *J Biol Chem* 1994; **269**: 19375–9.
22. Murphy G, Signer E. Bile acid metabolism in infants and children. *Gut* 1974; **15**: 151-163.
23. Strandvik B. Bile acid metabolism in low birthweight infants. *Acta Paediatr Scand Suppl* 1982; **296**: 71–4.
24. Jahnel J, Zöhrer E, Scharnagl H, Erwa W, Fauler G, Stojakovic T. Reference ranges of serum bile acids in children and adolescents. *Clin Chem Lab Med* 2015; **53**: 1807–13.
25. Suchy F, Balistreri W, Heubi J, Searcy J, Levin R. Physiologic cholestasis: Elevation of the primary serum bile acid concentrations in normal infants. *Gastroenterology* 1981; **80**: 1037–41.
26. Halilbasic E, Claudel T, Trauner M. Bile acid transporters and regulatory nuclear receptors in the liver and beyond. *J Hepatol* 2013; **58**: 155–68.
27. Dawson P, Lan T, Rao A. Bile acid transporters. *J Lipid Res* 2009; **50**: 2340–57.
28. Strautnieks S, Bull L, Knisely A, *et al.* A gene encoding a liver-specific ABC transporter is mutated in progressive familial intrahepatic cholestasis. *Nat Genet* 1998; **20**: 233–8.
29. Strautnieks S, Byrne J, Pawlikowska L, *et al.* Severe Bile Salt Export Pump Deficiency: 82 Different ABCB11 Mutations in 109 Families. *Gastroenterology* 2008; **134**: 1203–14.
30. Shaffer E. Review article: control of gall-bladder motor function. *Aliment Pharm Therap* 2000; **14**: 2–8.
31. Angelin B, Björkhem I, Einarsson K, Ewerth S. Hepatic uptake of bile acids in man. *J Clin Invest* 1982; **70**: 724–31.
32. Einarsson K, Reihnér E, Ewerth S, Björkhem I. Serum concentrations of unconjugated and conjugated cholic acid in portal venous and systemic venous blood of fasting man. *Scand J Clin Lab Invest* 2009; **49**: 83–91.

33. Song K, Ellis E, Strom S, Chiang J. Hepatocyte growth factor signaling pathway inhibits cholesterol 7α-hydroxylase and bile acid synthesis in human hepatocytes. *Hepatology* 2007; **46**: 1993–2002.
34. Grober J, Zaghini I, Fujii H, *et al.* Identification of a Bile Acid-responsive Element in the Human Ileal Bile Acid-binding Protein Gene. *J Biol Chem* 1999; **274**: 29749–54.
35. Plass JRM, Mol O, Heegsma J, *et al.* Farnesoid X receptor and bile salts are involved in transciptional regulation of the gene encoding the human bile salt export pump. *Hepatology* 2002; **35**: 589–96.
36. Denson L, Sturm E, Echevarria W, *et al.* The Orphan Nuclear Receptor, shp, Mediates Bile Acid-Induced Inhibition of the Rat Bile Acid Transporter, ntcp. *Gastroenterology* 2001; **121**: 140–7.
37. Seol W, Choi H, Moore D. Isolation of proteins that interact specifically with the retinoid X receptor: two novel orphan receptors. *Mol Endocrinol* 1995; **9**: 72–85.
38. Modica S, Gadaleta R, Moschetta A. Deciphering the nuclear bile acid receptor FXR paradigm. *Nucl Recept Signal* 2010; **8**: e005.
39. Schaap F, Trauner M, Jansen P. Bile acid receptors as targets for drug development. *Nat Rev Gastroentero* 2014; **11**: 55–67.
40. Parks DJ, Blanchard SG, Bledsoe RK, *et al.* Bile Acids: Natural Ligands for an Orphan Nuclear Receptor. *Science* 1999; **284**: 1365–8.
41. Hirschfield G, Mason A, Luketic V, *et al.* Efficacy of Obeticholic Acid in Patients With Primary Biliary Cirrhosis and Inadequate Response to Ursodeoxycholic Acid. *Gastroenterology* 2015; **148**: 751–761.
42. Seol W, Choi H-S, Moore DD. An orphan nuclear hormone receptor that lacks a DNA binding domain and heterodimerizes with other receptors. *Science* 1996; **272**: 1336–9.
43. Neimark E, Chen F, Li X, Shneider B. Bile acid–induced negative feedback regulation of the human ileal bile acid transporter. *Hepatology* 2004; **40**: 149–56.
44. Lu T, Makishima M, Repa J, *et al.* Molecular basis for feedback regulation of bile acid synthesis by nuclear receptors. *Mol Cell* 2000; **6**: 507–15.
45. Abrahamsson A, Gustafsson U, Ellis E, *et al.* Feedback regulation of bile acid synthesis in human liver: Importance of HNF-4α for regulation of CYP7A1. *Biochem Bioph Res Commun* 2005; **330**: 395–9.
46. Kerr TA, Saeki S, Schneider M, *et al.* Loss of nuclear receptor SHP impairs but does not eliminate negative feedback regulation of bile acid synthesis. *Dev Cell* 2002; **2**: 713–20.
47. Wang L, Lee Y-K, Bundman D, *et al.* Redundant pathways for negative feedback regulation of bile acid production. *Dev Cell* 2002; **2**: 721–31.

48. Itoh N. Hormone-like (endocrine) Fgfs: their evolutionary history and roles in development, metabolism, and disease. *Cell Tissue Res* 2010; **342**: 1–11.
49. Maddaluno L, Urwyler C, Werner S. Fibroblast growth factors: key players in regeneration and tissue repair. *Development* 2017; **144**: 4047–60.
50. Beenken A, Mohammadi M. The FGF family: biology, pathophysiology and therapy. *Nat Rev Drug Discov* 2009; **8**: 235–53.
51. Goetz R, Beenken A, Ibrahimi O, *et al.* Molecular Insights into the Klotho-Dependent, Endocrine Mode of Action of Fibroblast Growth Factor 19 Subfamily Members. *Mol Cell Biol* 2007; **27**: 3417–28.
52. Kurosu H, Choi M, Ogawa Y, *et al.* Tissue-specific Expression of βKlotho and Fibroblast Growth Factor (FGF) Receptor Isoforms Determines Metabolic Activity of FGF19 and FGF21. *J Biol Chem* 2007; **282**: 26687–95.
53. Lin BC, Wang M, Blackmore C, Desnoyers LR. Liver-specific activities of FGF19 require Klotho beta. *J Biol Chem* 2007; **282**: 27277–84.
54. Xie M-H, Holcomb I, Deuel B, *et al.* FGF-19, a novel fibroblast growth factor with unique specificity for FGFR4. *Cytokine* 1999; **11**: 729–35.
55. Zweers SJL., Booij KAC, Komuta M, *et al.* The human gallbladder secretes fibroblast growth factor 19 (FGF19) into bile: towards defining the role of FGF19 in the enterobiliary tract. *Hepatology* **55**: 575–83.
56. Angelin B, Larsson TE, Rudling M. Circulating Fibroblast Growth Factors as metabolic regulators—a critical appraisal. *Cell Metab* 2012; **16**: 693–705.
57. Tomlinson E, Fu L, John L, *et al.* Transgenic mice expressing human fibroblast growth factor-19 display increased metabolic rate and decreased adiposity. *Endocrinology* 2002; **143**: 1741–7.
58. Fu L, John L, Adams S, *et al.* Fibroblast Growth Factor 19 Increases Metabolic Rate and Reverses Dietary and Leptin-Deficient Diabetes. *Endocrinology* 2004; **145**: 2594–603.
59. Kir S, Beddow S, Samuel V, *et al.* FGF19 as a postprandial, insulin-independent activator of hepatic protein and glycogen synthesis. *Science* 2011; **331**: 1621–4.
60. Potthoff M, Boney-Montoya J, Choi M, *et al.* FGF15/19 Regulates Hepatic Glucose Metabolism by Inhibiting the CREB-PGC-1α Pathway. *Cell Metab* 2010; **13**: 729-38.
61. Nishimura T, Utsunomiya Y, Hoshikawa M, Ohuchi H, Itoh N. Structure and expression of a novel human FGF, FGF-19, expressed in the fetal brain. *Biochim Biophys Acta* 1999; **1444**: 148–51.

62. Wright T, Ladher R, McWhirter J, Murre C, Schoenwolf G, Mansour S. Mouse FGF15

is the ortholog of human and chick FGF19, but is not uniquely required for otic induction. *Dev Biol* 2004; **269**: 264–75.
63. Potthoff M, Kliewer S, Mangelsdorf D. Endocrine fibroblast growth factors 15/19 and 21: from feast to famine. *Genes Dev* 2012; **26**: 312–24.
64. Song K, Li T, Owsley E, Strom S, Chiang JY. Bile acids activate Fibroblast Growth Factor 19 signaling in human hepatocytes to inhibit cholesterol 7α-hydroxylase gene expression. *Hepatology* 2009; **49**: 297–305.
65. Holt JA, Luo G, Billin AN, *et al.* Definition of a novel growth factor-dependent signal cascade for the suppression of bile acid biosynthesis. *Genes Dev* 2003; **17**: 1581–91.
66. Inagaki T, Choi M, Moschetta A, *et al.* Fibroblast Growth Factor 15 functions as an enterohepatic signal to regulate bile acid homeostasis. *Cell Metab* 2005; **2**: 217–25.
67. Pandak W, Heuman D, Hylemon P, Chiang J, Vlahcevic Z. Failure of intravenous infusion of taurocholate to down-regulate cholesterol 7α-hydroxylase in rats with biliary fistulas. *Gastroenterology* 1995; **108**: 533–44.
68. Yu C, Wang F, Kan M, *et al.* Elevated cholesterol metabolism and bile acid synthesis in mice lacking membrane tyrosine kinase receptor FGFR4. *J Biol Chem* 2000; **275**: 15482–9.
69. Schaap FG, van der Gaag NA, Gouma DJ, Jansen PL. High expression of the bile salt-homeostatic hormone Fibroblast Growth Factor 19 in the liver of patients with extrahepatic cholestasis. *Hepatology* 2009; **49**: 1228–35.
70. Lundåsen T, Gälman C, Angelin B, Rudling M. Circulating intestinal Fibroblast Growth Factor 19 has a pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med* 2006; **260**: 530–6.
71. Al-Khaifi A, Straniero S, Voronova V, *et al.* Asynchronous rhythms of circulating conjugated and unconjugated bile acids in the modulation of human metabolism. *J Intern Med* 2018; **284**: 546–59.
72. Johansson H, Mörk L-M, Li M, *et al.* Circulating Fibroblast Growth Factor 19 in Portal and Systemic Blood. *J Clin Exp Hepatol* 2018; **8**: 162-8.
73. Gälman C, Angelin B, Rudling M. Pronounced variation in bile acid synthesis in humans is related to gender, hypertriglyceridaemia and circulating levels of fibroblast growth factor 19. *J Intern Med* 2011; **270**: 580–8.
74. Goodwin B, Watson MA, Kim H, Miao J, Kemper JK, Kliewer SA. Differential regulation of rat and human CYP7A1 by the nuclear oxysterol receptor Liver X receptor-α. *Mol Endocrinol* 2003; **17**: 386–94.
75. Kotokorpi P, Ellis E, Parini P, *et al.* Physiological Differences between Human and Rat Primary Hepatocytes in Response to Liver X Receptor Activation by 3-[3-[N-(2-Chloro-3-

trifluoromethylbenzyl)-(2,2-diphenylethyl)amino]propyloxy]phenylacetic Acid Hydrochloride (GW3965). *Mol Pharmacol* 2007; **72**: 947–55.
76. Li J, Dawson P. Animal models to study bile acid metabolism. *Biochim Biophys Acta - Mol Bas Dis* 2018; **1865**: 895–911.
77. Liu, Lu, Lu -F, *et al.* Potency of Individual Bile Acids to Regulate Bile Acid Synthesis and Transport Genes in Primary Human Hepatocyte Cultures. *Toxicol Sci* 2014; **141**: 538–46.
78. Wagner M, Zollner G, Trauner M. New molecular insights into the mechanisms of cholestasis. *J Hepatol* 2009; **51**: 565–80.
79. Chen H-L, Liu Y-J, Chen H-L, *et al.* Expression of hepatocyte transporters and nuclear receptors in children with early and late-stage biliary atresia. *Ped Res* 2008; **63**: 667–73.
80. Wunsch E, Milkiewicz M, Wasik U, *et al.* Expression of hepatic Fibroblast Growth Factor 19 is enhanced in Primary Biliary Cirrhosis and correlates with severity of the disease. *Sci Rep* 2015; **5**: 13462.
81. Johansson H, Svensson J, Almström M, *et al.* Regulation of bile acid metabolism in biliary atresia: Reduction of FGF19 by Kasai portoenterostomy and possible relation to early outcome. *J Intern Med* 2020 doi: 10.1111/joim.13028. Epub ahead of print.
82. Choi M, Moschetta A, Bookout AL, *et al.* Identification of a hormonal basis for gallbladder filling. *Nat Med* 2006; **12**: 1253–5.
83. Barrera F, Azocar L, Molina H, *et al.* Effect of cholecystectomy on bile acid synthesis and circulating levels of fibroblast growth factor 19. *Ann Hepatol* 2015; **14**: 710–21.
84. Park S, Kim W, Bae I-H, *et al.* Common bile duct dilatation after cholecystectomy: a one-year prospective study. *J Korean Surg Soc* 2012; **83**: 97–101.
85. Pakarinen M, Johansen L, Svensson J, *et al.* Outcomes of biliary atresia in the Nordic countries – A multicenter study of 158 patients during 2005–2016. *J Pediatr Surg* 2017; **53**: 1509–15.
86. Hartley JL, Davenport M, Kelly DA. Biliary Atresia. *Lancet* 2009; **374**: 1704–13.
87. Fischler B, Haglund B, Hjern A. A population-based study on the incidence and possible pre- and perinatal etiologic risk factors of biliary atresia. *Journal Pediatr* 2002; **141**: 217–22.
88. Mezina A, Karpen S. Genetic contributors and modifiers of biliary atresia. *Dig Dis* 2015; **33**: 408–14.
89. Feldman A, Mack C. Biliary Atresia. *J Pediatr Gastr Nutr* 2015; **61**: 167–75.

90. Kasai M, Kimura S, Asakura Y, Suzuki H, Taira Y, Ohashi E. Surgical treatment of

biliary atresia. *J Pediatr Surg* 1968; **3**: 665–75.
91. Sokol R, Shepherd R, Superina R, Bezerra J, Robuck P, Hoofnagle J. Screening and outcomes in biliary atresia: Summary of a National Institutes of Health workshop. *Hepatology* 2007; **46**: 566–81.
92. Berry M, Friend D. High-yield preparation of isolated rat liver parenchymal cells A Biochemical and Fine Structural Study. *J Cell Biol* 1969; **43**: 506–20.
93. Strom SC, Jirtle RL, Jones RS, *et al.* Isolation, culture, and transplantation of human hepatocytes. *J Natl Cancer Inst* 1982; **68**: 771–8.
94. Björkhem I, Falk O. Assay of the major bile acids in serum by isotope dilution-mass spectrometry. *Scand J Clin Lab Invest* 1983; **43**: 163–70.
95. Dumaswala R, Berkowitz D, Setchell KD., Heubi JE. Effect of fasting on the enterohepatic circulation of bile acids in rats. *Am J Physiol* 1994; **267**: 836–42.
96. Kong B, Guo G. Enhanced In Vitro Refolding of Fibroblast Growth Factor 15 with the Assistance of SUMO Fusion Partner. *Plos One* 2011; **6**: e20307.
97. Rowland M. Influence of Route of Administration on Drug Availability. *J Pharm Sci* 1972; **61**: 70–4.
98. Meier JJ, Veldhuis JD, Butler PC. Pulsatile insulin secretion dictates systemic insulin delivery by regulating hepatic insulin extraction in humans. *Diabetes* 2005; **54**: 1649–56.
99. Hofmann A, Hagey L. Key discoveries in bile acid chemistry and biology and their clinical applications: history of the last eight decades. *J Lipid Res* 2014; **55**: 1553–95.
100. Katafuchi T, Esterházy D, Lemoff A, *et al.* Detection of FGF15 in Plasma by Stable Isotope Standards and Capture by Anti-peptide Antibodies and Targeted Mass Spectrometry. *Cell Metab* 2015; **21**: 898–904.
101. Montagnani M, Matvey , Flavia , *et al.* A New Model for Portal Protein Profile Analysis in Course of Ileal Intraluminal Bile Acid Infusion Using an In Situ Perfused Rat Intestine. *Med Chem* 2011; **7**: 257–64.
102. Koelfat K, Bloemen J, Jansen P, Dejong C, Schaap F, Damink S. The portal-drained viscera release fibroblast growth factor 19 in humans. *Physiol Rep* 2016; **4**: e13037.
103. Scott RB, Strasberg SM, El-Sharkawy TY, Diamant NE. Regulation of the Fasting Enterohepatic Circulation of Bile Acids by the Migrating Complex in Dogs. *J Clin Invest* 1983; **71**: 644–54.
104. Abukawa D, Nakagawa M, Iinuma K, No M, Ohi R, Goto J. Hepatic and serum bile acid compositions in patients with Biliary atresia: A microanalysis using Gas Chromatography-Mass Spectrometry with negative ion chemical ionization detection. *J Exp Med* 1998; **185**: 227–37.

105. Zhou M, Learned R, Rossi S, DePaoli A, Tian H, Ling L. Engineered FGF19 eliminates bile acid toxicity and lipotoxicity leading to resolution of steatohepatitis and fibrosis in mice. *Hepatol Commun* 2017; **1**: 1024–42.
106. Zhou M, Learned R, Rossi S, DePaoli A, Tian H, Ling L. Engineered fibroblast growth factor 19 reduces liver injury and resolves sclerosing cholangitis in Mdr2-deficient mice. *Hepatology* 2016; **63**: 914–29.
107. Mayo MJ, Wigg AJ, Leggett BA, *et al.* NGM282 for Treatment of Patients With Primary Biliary Cholangitis: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial. *Hepatol Commun* 2018; **2**: 1037–50.
108. Luo J, Ko B, Elliott M, *et al.* A nontumorigenic variant of FGF19 treats cholestatic liver diseases. *Sci Transl Med* 2014; **6**: 1-11.
109. Zhou M, Wang X, Phung V, *et al.* Separating Tumorigenicity from Bile Acid Regulatory Activity for Endocrine Hormone FGF19. *Cancer Res* 2014; **74**: 3306–16.
110. Trauner M, Fuchs CD, Halilbasic E, Paumgartner G. New therapeutic concepts in bile acid transport and signaling for management of cholestasis. *Hepatology* 2017; **65**: 1393–404.
111. French D, Lin B, Wang M, *et al.* Targeting FGFR4 Inhibits Hepatocellular Carcinoma in Preclinical Mouse Models. *PLoS ONE* 2012; **7**: e36713.
112. Miura S, Mitsuhashi N, Shimizu H, *et al.* Fibroblast growth factor 19 expression correlates with tumor progression and poorer prognosis of hepatocellular carcinoma. *BMC Cancer* 2012; **12**: 56–70.
113. Jansen PLM, Werven J, Aarts E, *et al.* Alterations of Hormonally Active Fibroblast Growth Factors after Roux-en-Y Gastric Bypass Surgery. *Dig Dis* 2011; **29**: 48–51.
114. Sánchez-Infantes D, Gallego-Escuredo J, Díaz M, *et al.* Circulating FGF19 and FGF21 surge in early infancy from infra- to supra-adult concentrations. *Int J Obes* 2015; **39**: 742–6.
115. Zhou K, Wang J, Xie G, *et al.* Distinct Plasma Bile Acid Profiles of Biliary Atresia and Neonatal Hepatitis Syndrome. *J Proteome Res* 2015; **14**: 4844–50.
116. Wu A-L, Coulter S, Liddle C, *et al.* FGF19 Regulates Cell Proliferation, Glucose and Bile Acid Metabolism via FGFR4-Dependent and Independent Pathways. *PLoS ONE* 2011; **6**: e17868.

PUBLICATIONS NOT INCLUDED IN THIS THESIS

Tokodai K, Kumagai-Braesch M, Karadagi A, Johansson H, Ågren N, Jorns C, Ericzon B-G, Ellis E. Blood Group Antigen Expression in Isolated Human Liver Cells in Preparation for Implementing Clinical ABO-Incompatible Hepatocyte Transplantation. *Journal of Clinical and Experimental Hepatology.* 2020; **10**: 106-113.

Santinha D, Klopot A, Marques I, Ellis E, Jorns C, Johansson H, Melo T, Antonson P, Jakobsson T, Félix V, Gustafsson J-Å, Domingues M, Mode A, Helguero L. Lipidomic analysis of human primary hepatocytes following LXR activation with GW3965 identifies AGXT2L1 as a main target associated to changes in phosphatidylethanolamine. *The Journal of Steroid Biochemistry and Molecular Biology.* 2020; **198**: 105558.

Gnocchi D, Ellis E, Johansson H, Eriksson M, Bruscalupi G, Steffensen K, Parini P. Diiodothyronines regulate metabolic homeostasis in primary human hepatocytes by modulating mTORC1 and mTORC2 activity. *Molecular and Cellular Endocrinology.* 2020; **499**: 110604.

Cornillet M, Jansson H, Schaffer M, Hertwig L, Berglin L, Zimmer C, Johansson H, Ellis E, Isaksson B, Gonzalez-Galarza F, Middleton D6, Malmberg K-J, Sparrelid E, Björkström N. Imbalance of Genes Encoding Natural Killer Immunoglobulin-Like Receptors and Human Leukocyte Antigen in Patients With Biliary Cancer. *Gastroenterology.* 2019; **157**: 1067-80.

Sparrelid E, Johansson H, Gilg S, Nowak G, Ellis E, Isaksson B. Serial Assessment of Growth Factors Associated with Liver Regeneration in Patients Operated with Associating Liver Partition and Portal Vein Ligation for Staged Hepatectomy. *European Surgical Research*. 2018; **59**: 72-82.

Karadagi A, Johansson H, Zemack H, Salipalli S, Mörk L-M, Kannisto K, Jorns C, Gramignoli R, Strom S, Stokkeland K, Ericzon B-G, Jonigk D, Janciauskiene S, Nowak G, Ellis E. Exogenous alpha 1-antitrypsin down-regulates SERPINA1 expression. *Plos One.* 2017; **12**: e0177279.

Watanabe M, Zemack H, Johansson H, Hagbard L, Jorns C, Li M, Ellis E. Maintenance of Hepatic Functions in Primary Human Hepatocytes Cultured on Xeno-Free and Chemical

Defined Human Recombinant Laminins. *Plos One*. 2016; **11**: e0161383.

Li M, Ellis E, Johansson H, Nowak G, Isaksson B, Gnocchi D, Parini P, Axelsson J. Changes in gluconeogenesis and intracellular lipid accumulation characterize uremic human hepatocytes ex vivo. The American Journal of Physiology: Gastrointestinal and Liver Physiology. 2016; **310**: G952-61.

Johansson H, Ellis E. Assay of Bile Acid Conjugation and Excretion in Human Hepatocytes. In: Protocols in *In Vitro* Hepatocyte Research. 2015: 323-331.

Jorns C, Nowak G, Nemeth A, Zemack H, Mörk L-M, Johansson H, Gramignoli R, Watanabe M, Karadagi A, Alheim M, Hauzenberger D, van Dijk R, Bosma P.J, Ebbesen F, Szakos A, Fischler B, Strom S, Ellis E, Ericzon B-G. De Novo Donor-Specific HLA Antibody Formation in Two Patients With Crigler-Najjar Syndrome Type I Following Human Hepatocyte Transplantation With Partial Hepatectomy Preconditioning. *American Journal of Transplantation.* 2015; **16**: 1021-30.

Marquardt N, Béziat V, Nyström S, Hengst J, Ivarsson M, Kekäläinen E, Johansson H, Mjösberg J, Westgren M, Lankisch T, Wedemeyer H, Ellis E, Ljunggren H-G, Michaëlsson J, Björkström N. Cutting Edge: Identification and Characterization of Human Intrahepatic CD49a+ NK Cells. *The Journal of Immunology*. 2015; **6**: 2467-71.

L. Berglin, Berquist A, Johansson H, Glaumann H, Jorns C, Lunemann S, Wedemeyer H, Ellis E, Björkström N. In Situ Characterization of Intrahepatic Non-Parenchymal Cells in PSC Reveals Phenotypic Patterns Associated with Disease Severity. *Plos One*. 2014; **9**: e105375.